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OF THE NEW YORK ACADEMY OF MEDICINE

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by

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and

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BULLETIN OF
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JANUARY 1949

ADVANCES IN THERAPY*

GEORGE BAEHR

THIS is the first Graduate Fortnight of the Academy devoted to a general review of recent Advances in Therapy. The preceding twenty annual Graduate Fortnights have each been devoted to a consideration of the diseases of one anatomical system, such as the cardiovascular or to one specific field of medicine such as the infections or neoplasms. In breaking with tradition, the Committee on Medical Education of the Academy and its subcommittees on the Graduate Fortnight have been moved by the remarkably rapid advances in the field of therapy within the last decade, which have left large gaps in the knowledge of all physicians who received their undergraduate and post-graduate training in medicine even as recently as ten or twenty years ago.

During my own early years of medical practice, therapy other than surgical was largely palliative or psychological or directed to the alleviation of symptoms. Only a half dozen diseases yielded to specific forms of therapy and these were crude and imperfect. Empiricism was still the order of the day. There is small wonder that many of us, following the lead of Skoda of an earlier and still more empirical gen-

* Opening address, 21st Graduate Fortnight of The New York Academy of Medicine, October 4, 1948 by the President of the Academy.

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eration, were therapeutical nihilists who found it more fruitful to concentrate our efforts upon refinements in medical diagnosis and on the laboratory techniques of bacteriology, pathology and biochemistry which were aids to a better understanding of fundamental disease processes. In the wards and laboratories of the hospitals we sought to improve the accuracy of our diagnosis and tried to unravel the mysteries of etiology and pathogenesis.

As I look back over those days, I appreciate that we were laying the groundwork of medicine in preparation for the new era of therapy in which we are now entering. And yet, I must admit, that our intense concentration upon these fundamentals was in part an escape from that discouraging sense of therapeutic helplessness and blank futility which came over us daily as we moved from bed to bed and confessed our inability to end or even shorten most of the diseases which confronted us.

How the picture has changed in an incredibly short time! The great advances in therapy which will be reviewed during this Fortnight are only a forecast of more and better therapeutic discoveries in the immediate future. A new vista of therapeutics has been revealed to us in the last decade or two through discovery of the antibiotics, the anticoagulants, the antihistamine drugs, the new and powerful hormonal agents with which the physiology of all organs and tissues of the body can be modified, the new knowledge of the amino acids, minerals and vitamins which give us the means to maintain or restore the nutritional state of the body in health and disease, the new radioactive materials. These are a few of the additions to our therapeutic armamentarium which are responsible for a significant and progressive reduction in mortality and an abbreviation of morbidity.

The evening lectures of the Fortnight, the morning discussion panels, the afternoon clinical demonstrations at the great hospitals of the city and the remarkable teaching exhibit which has been assembled at the Academy, provide an unrivalled opportunity for all members of the medical profession to bring their knowledge of modern therapeutics up to date, so that they may serve the people in accordance with the best modern standards and prepare themselves for even greater advances in therapy which are yet to come.

THE USE OF ANTIEPILEPTIC DRUGS*

H. Houston Merritt

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THE USE OF anticonvulsant drugs is only one part of the treatment of patients with convulsive seizures but at the present time, they are the most important single factor in the treatment of such patients. Success or failure in the treatment of patients with seizures depends almost wholly on the control of the attacks. In the vast majority of the cases, anticonvulsant drugs are the only means at our disposal which will prevent or lessen the frequency of seizures.

The anticonvulsant drugs which are in use at the present time can not be considered as a cure for epilepsy. Although very little is known in regard to their mode of action, it is apparent that for the most part they serve only to alter the activity of the cerebral cortex in some manner so that the tendency for the occurrence of convulsive seizures is diminished. They have very little, if any, permanent effect on the underlying physico-chemical disturbance which is responsible for seizures since cessation of a treatment, which is effective in the prevention of seizures, almost regularly results in the prompt recurrence of attacks.

In recent years there has been a revival of interest in the medical therapy of epilepsy and new drugs are being tested in various clinics throughout the country. The direct result of these studies is the introduction of more effective remedies for the prevention of attacks. Of greater importance, however, for the ultimate control of the disease is the possibility that the studies in regard to the nature of the chemical compounds, which inhibit seizures, will throw some light on the nature of the fundamental pathological physiology of the disease.

Classification of Patients with Seizures: The intelligent use of anticonvulsant drugs is dependent to a great extent on a knowledge of the type or types of seizure to which the patient is subject. This requires a brief discussion of the classification of patients with convulsive seizures.

* Presented October 5, 1948 before the 21st Graduate Fortnight of The New York Academy of Medicine. From the Department of Neurology, College of Physicians and Surgeons, Columbia University, and the Neurological Institute, New York.

There are three ways of subdividing the patients with epilepsy, all of which have some value.

The first method divides the cases into two groups, symptomatic and idiopathic, according to the presence or absence of an organic lesion. This division emphasizes the fact that a thorough study of each case is a necessary preliminary to the application of the therapy. The concept of symptomatic and idiopathic epilepsy is a fallacious one, however, if we assume that it gives us any understanding of the underlying pathophysiology of the disease. It is evident that the seizures of patients with symptomatic epilepsy are not due solely to the organic lesion in the central nervous system. This lesion is constantly present but the attacks are irregular in occurrence. On the other hand, the methods of study which are now available do not indicate whether or not there is a structural or functional abnormality of the nervous system in the patients with so-called idiopathic epilepsy. Studies of the electrical activity of the brain by the method of electroencephalography[1] almost regularly show evidence of a disturbance of the cortical activity in the moments preceding and during a seizure. In addition abnormal cortical activity can be found in the interval between attacks in a large percentage of the patients.

The second method[2] of dividing the cases is based to a great extent on the result of the electroencephalographic study of the patient. The occurrence of focal or generalized seizures is dependent on whether the abnormal discharge which initiates the seizure is generalized or localized to one particular region of the cerebrum. This method of separating the attacks is of special value to the neurosurgeon, who approaches the treatment of epilepsy from the surgical viewpoint. It is also of some value to the clinician in the choice of various treatment methods.

The third method of dividing the cases of epilepsy which is based on the clinical manifestations of the seizure is of particular value in the selection of the drug to be used in the therapy. Epileptic attacks may be divided roughly into three groups. Some patients may have only one type of seizure but it is not uncommon for the afflicted individual to suffer with two or even three types of spells. The three types of attacks are 1) petit mal, 2) grand mal (including Jacksonian and focal seizures), and 3) psychic equivalents or psychomotor attacks.

Petit mal seizures, which occur predominantly in children, are manifested by a transient clouding of consciousness lasting for only a few

seconds, with or without minor movements of the head, eyes, and extremities. Other features of petit mal epilepsy include transient loss of postural tone (cataplexy), myoclonic jerks, and the characteristic changes in the electroencephalogram. The phenomena which occur in a grand mal attack may be quite varied. Characteristically these attacks are ushered in by a warning (aura) and are followed by a sudden loss of consciousness with tonic-clonic spasms of the musculature, with or without urinary and fecal incontinence. The convulsive movements continue for one to many minutes and are often followed by sleep or a brief period of confusion. Jacksonian seizures are characterized by the occurrence of clonic movements of the muscles of one extremity or one-half of the face without the loss of consciousness. The clonic movements may spread from one extremity to the other or to the face. If the movements spread to the opposite half of the body, consciousness is lost and the further course of the seizure is similar to that of the usual grand mal attacks. Focal seizures are grand mal attacks initiated by some sensory or motor phenomenon which can be referred to excitation of some specific region of the brain. Psychic equivalents or psychomotor attacks are terms used to describe a heterogeneous group of epileptiform disturbances with clinical manifestations which do not conform to those of the grand mal or petit mal types of seizures. The common form of psychomotor attack is often confused with petit mal. It differs from the latter in that the duration of the period of mental cloudiness is longer and the range of muscular movements is greater, and it is distinguished from a grand mal seizure by the fact the patient does not fall to the ground in a tonic-clonic seizure with complete loss of consciousness. Rarely psychic equivalent attacks may be manifested by a clouding of the mental state for many hours during which time the patient may perform acts of which he is entirely unaware.

Historical Review of the Development of Antiepileptic Drugs: The modern approach to the treatment of epilepsy begins with the middle of the nineteenth century. While it is true that various drugs were used by the ancient and medieval physicians, the treatment methods of these times were mainly dietary restrictions, the invocation of supernatural powers and the administration of substances which had magical properties.[3]

The list of drugs which were used in the latter part of the nineteenth century[4] include the bromides, opium, codeine, borax, chloral

hydrate, amylene hydrate, nitroglycerin, chloretone, zinc salts, urethane, solanum carolinense, simulo, trional, iron, coal tar derivatives (phenacetin, antipyrin and acetanalid), and chloroform. The introduction of the bromides as a therapeutic agent in epilepsy resulted from an interesting observation of Locock[5] that the seizures in epilepsy were often related to hysteria or the menses. He prescribed bromides as a remedy in a case of epilepsy connected with sexual excitement and after all other medication had failed, the result had been an entire cessation of attacks. He had also tried the bromide of potassium in 14 or 15 similar cases, with failure in only one.

The next advance in the pharmacological treatment of epilepsy was the introduction of the use of luminal (phenobarbital) by Hauptmann in 1912.[6] This drug which had been introduced as a sedative and a hypnotic, was administered in daily doses of 0.3 gram to patients with frequent and severe grand mal seizures and favorable results were reported. There was a reduction in the frequency of the attacks and a decrease in the severity of the attacks that did occur, and general improvement in the health and nutrition of the patients who had suffered from over-dosage of bromides. He noted that sudden withdrawal of the drug would result in the precipitation of a number of seizures.

Between 1912 and 1937 researches in the therapy of epilepsy were directed toward the modification of the internal milieu by restriction of fluids and by dietary measures rather than by the administration of drugs. In 1921 Geyelin[7] demonstrated that fasting influenced the incidence of seizures in a certain number of the patients with epilepsy and suggested that the benefit was due to the acidosis induced by the fasting. This observation of Geyelin led to other methods of producing an acidosis. Wilder[8] at the Mayo Clinic demonstrated that a diet high in fat and extremely low in carbohydrate was as effective in producing acidosis as fasting. Wilder proposed the diet on the theory that acetoacetic acid should behave pharmacologically as an anesthetic. Other observers believed that a high fat diet owed its therapeutic value to its action on the acid-base equilibrium by correcting an abnormal tendency toward the spontaneous development of alkalosis.

Data which showed disturbance in the water-balance in patients with epilepsy were presented by Gamble[9] in 1923. McQuarrie[10] in 1929 demonstrated that a negative fluid balance tended to reduce the frequency of seizures. The greatest advocate of the dehydration therapy,

however. was Temple Fay[11] of Philadelphia. The excessive restriction of fluid intake necessary to maintain a negative fluid balance negated any wide acceptance of this form of treatment.

In 1937 a new method of testing the anticonvulsive activity of drugs in animals was devised by Putnam and Merritt.[12] By this method, Merritt, Putnam and Schwab tested a large number of compounds and found a number of them to have an anticonvulsant activity greater than those already in common use. One of these compounds, sodium diphenyl hydantoinate (phenytoin sodium, dilantin sodium, epanutin) was introduced into the treatment of epilepsy in 1938[13] The results obtained with sodium diphenyl hydantoinate has stimulated further search for more effective anti-convulsant drugs and in the past 10 years a number of new compounds have been developed.[14]

Use of Anticonvulsant Drugs: The drugs which are most commonly used at the present time in the treatment of patients with convulsive seizures are the bromides, barbiturates, hydantoins, and oxazolidinediones. Other drugs are being subjected to experimental use but reports in regard to their efficacy are not available.

The treatment of patients with convulsive seizures can not be standardized, since each patient presents an individual problem. Some generalization can be made regarding the drugs which are most likely to be effective in the various types of seizures and in regard to the dosage to be used. A point of practical importance is that a change should not be made abruptly from one form of therapy to another. Even though two drugs apparently have similar action, the sudden replacement of one of them by another may result in the occurrence of status epilepticus. For this reason the full dosage of the first medication should be continued for several days until an adequate reservoir of the new drug has been built up in the patient's system.

Grand Mal Seizures: Phenytoin sodium and phenobarbital are the most effective drugs in the treatment of patients with grand mal seizures. Either of these two drugs can be tried as the first method of therapy. If one proves ineffective or is not tolerated, the other can be substituted or a combination of the two can be given together. Occasionally, 3-methyl-5,5 phenylethyl hydantoin (Mesantoin) or a combination of this drug with the phenytoin sodium will give the best results. Rarely the bromides or a combination of bromides and phenobarbital or phenytoin sodium will be most effective.

Petit Mal Attacks: Only rarely are any of the drugs, which are effective in the treatment of grand mal, of any value in the treatment of patients with petit mal attacks. Trimethyloxazolidinedione (tridione) is the drug of choice for the latter type of seizure. Other oxazolidinediones have been used experimentally but the reports are inadequate for satisfactory evaluation of their efficacy.

Psychomotor Attacks: The drugs which are effective in the treatment of grand mal seizures are effective in the treatment of patients with attacks of the psychomotor type. Larger dosages are required and the results are on the whole not as good as they are in the patients with grand mal seizures.

Dosages and Side-Effects of the Various Drugs. Bromides: Although any of the salts may be used, the drug is most commonly given as the sodium or potassium salt, in tablets, or aqueous solution. The average dose for an adult is 15 grains (1 gm.) three times daily with proportionate doses to children according to size. In the absence of toxic symptoms this dose can be increased to a maximum of 30 grains (2 gm.) three times daily. The chloride intake must be kept at an adequate level to prevent undue replacement of chloride ion in the body fluid by the bromide. Facilities for the determination of the bromide content of the serum should be available. The effective level may be as low as 100 mg. per hundred cubic centimeters in some patients, whereas 300 mg. per hundred cubic centimeters may not be effective in others. Toxic symptoms may develop with a concentration of 150 mg. or greater. The chief objections to the use of bromides lie in the frequency of the development of skin rash, toxic psychosis, and their reputed tendency to produce mental dullness.

Phenobarbital (Phenylethyl barbituric acid): For the average adult the initial dose of phenobarbital should be 1½ grains daily. This can be given at bed time. If after a trial period of two weeks or as long as is necessary to determine whether this dose is effective, further increases can be made in the dosage until the patient is taking as much as 4½ to 6 grains per day. If this amount of the drug is not sufficient to control the seizure, it is probable that a further increase will not be of value. In children, the dose of phenobarbital should be in proportion to weight, but it has been found that children are able to tolerate and require almost as large a dose as adults. It is, therefore, advisable to give children over 6 or 7 years of age the minimum dose of 1½ grains per day. The

toxic symptoms of phenobarbital are well known and need no further discussion. Drowsiness is common at the start of the treatment but this may disappear with continued use of the medicine. Allergic skin rashes are infrequent and there are no effects on the hemopoietic system.

Mebaral: Mebaral, the 3 methyl derivative of phenylethyl barbituric acid (phenobarbital) can be used as a substitute for the latter because it has slightly less sedative effect. Approximately twice the dosage of phenobarbital is required and at this dosage the sedative effect is similar to that of phenobarbital.

Phenytoin Sodium: Phenytoin sodium (dilantin sodium), 5,5-diphenyl glycolyl urea is particularly valuable in the treatment of psychomotor and grand mal attacks. This drug has the advantage over phenobarbital and the bromides in that it has very little or no hypnotic activity. The regulation of the dosage is more difficult and minor toxic symptoms are more frequent. The toxic symptoms are not serious and it is almost impossible for a patient to take a fatal dose of the medicine.

The principle of administration of phenytoin sodium is similar to that of phenobarbital, that is, the establishment and maintenance of a reservoir of the drug sufficient to control the seizures. In the average adult, the initial dose should be 4½ grains (0.3 gm.) daily. If any seizures occur after two weeks of this dosage, it should be increased to 6 grains (0.4 gm.) daily. Further increases in the dosage should be by increments of 1½ grains (0.1 gm.) until the maximum dose of 9 grains (0.6 gm.) daily is reached. In the majority of adults, 6 grains (0.4 gm.) is the optimum dose. In children over 12 or 14 years, the average dose is 4½ grains to 6 grains (0.3 to 0.4 gm.) and in younger children 3 to 4½ grains (0.2 to 0.3 gm.). The medicine can be given in divided doses spread out through the day, or it can be given all in one dose at bed time. The drug is quite alkaline and it may cause gastric upsets. This can be prevented by giving the drug along with the meal or with some food.

The toxic symptoms of phenytoin sodium are different from those of phenobarbital in that nervousness or sleeplessness, rather than drowsiness, is more commonly an early symptom. Other toxic symptoms are gastric distress, nausea and vomiting, unsteadiness of gait, hypertrophy of the gums and dermatitis.

The minor toxic symptoms are frequently transient and may disappear with continuation of the therapy or when the dosage is tempo-

rarily reduced. Nystagmus, unsteadiness of gait and tremors of the extremities can be produced in practically all patients if the dosage is raised sufficiently high. A few adults will tolerate as much as 9 to 12 grains (0.6 to 0.8 gm.) but symptoms usually develop when the dose is increased beyond 7½ grains (0.5 gm.) The appearance of toxic symptoms calls for a temporary or permanent reduction of the dosage. If the reduced dose is not effective in controlling the seizures and attempts to increase the dose again result in the appearance of toxic symptoms, a combination of phenytoin sodium and phenobarbital or bromides should be tried, or one of the other hydantoins, such as mesantoin, should be substituted.

Gastric discomfort, nausea, and vomiting may be controlled by the administration of the drug along with a little bicarbonate of soda or at meal time. Dermatitis of a scarlatiniform or morbilliform nature occurs within two weeks of institution of therapy in approximately 5 to 10 per cent of the patients and is accompanied by fever and an eosinophilia. The rash usually disappears within a few days after withdrawal of the drug. Recurrence of the rash when treatment is reinstituted or the development of an exfoliative dermatitis precludes further use of the medicine.

The most troublesome toxic symptom of the drug is hypertrophy of the gums. This is most common in children and varies from a slight swelling of the gums to a marked hyperplasia with almost a total covering of the teeth. The hyperplastic tissue is usually quite firm without any tendency to bleeding. The swelling of the gum tissue is not related to any disturbance in the absorption or utilization of vitamin C. This hyperplasia can be retarded by daily massage of the gums. Excessive growth of the gum tissue can be excised by the electric cautery. The development of psychotic symptoms in patients under therapy with phenytoin sodium is rare and it is usually not possible to determine whether these symptoms are related to the use of the drug or not. In such cases a change in the type of treatment should be tried.

Other Hydantoins: Various derivatives of the hydantoins have anticonvulsive properties. In some patients one of these hydantoin derivatives may be more effective in controlling grand mal or psychomotor seizures than either phenobarbital or phenytoin sodium. The work on the majority of these drugs is still in the experimental stage, with the exception of the 3-methyl, 5-phenyl, 5-ethyl derivative, which has been

given an extensive clinical trial in several clinics. The average dose for children is 0.4 gm. daily, and for adults, 0.6 gm. The maximum daily dose is 1.0 gm. The drug can be used in combination with phenytoin sodium. The most common symptom of overdose is drowsiness. Toxic skin rashes are slightly more frequent than with the use of phenytoin sodium. Blood dyscrasias have occurred in a few patients who were receiving mesantoin and other anticonvulsant drugs. It is not definitely known whether mesantoin alone has any serious toxic effect on the blood forming organs.

Tridione (3,5,5-trimethyloxazolidine-2,4 dione): Tridione is the most effective drug in the treatment of petit mal seizures. The use of this drug is accompanied by a cessation or reduction in frequency of petit mal attacks in approximately 50 per cent of the cases. In a few patients the cessation of attacks is accompanied by a decrease in the abnormalities in the electroencephalogram. In such patients it is sometimes possible to discontinue the use of the drug without recurrence of the petit mal seizures. The drug is of no value in the control of grand mal or psychomotor seizures. If patients are subject to one of the latter types of seizures as well as petit mal, phenytoin sodium or phenobarbital should be given along with the tridione.

The dosage of tridione for the treatment of petit mal varies from 0.3 to 2.0 gm. daily, starting with 0.3 gm. and gradually increasing the dose until the seizures are controlled or toxic symptoms appear. Among the toxic symptoms are skin rashes, which require a cessation of the treatment, and visual symptoms—an unusual sensitivity to light. This latter symptom is apt to develop in adolescent or adult patients and is uncommon in young children. The photophobia is not accompanied by any change in visual acuity, and it disappears when the medicine is discontinued.

Several cases of fatal aplastic anemia[15] following the exhibition of tridione for periods of six and ten months have been reported. Prolonged use of the drug may also be accompanied by a decrease in the percentage of polymorphonuclear leukocytes in the blood without an absolute decrease in the total number of leukocytes. Although it is not known whether the observation of any precautions will make it possible to prevent serious or fatal changes in the blood, it is recommended that routine blood count be made monthly in patients using tridione. The drug should be discontinued if any significant changes are found.

Combination of Drugs: Since phenytoin sodium has very little sedative effect, it is particularly adapted to use in combination with phenobarbital, the bromides, or mesantoin. Various combinations can be used when one drug is not effective in controlling the seizures or when the effective dose of phenytoin sodium alone produces toxic symptoms. The doses of the combination must be worked out according to the tolerance of each patient. Three to five doses a day of a combination of 1½ grains (0.1 gm.) phenytoin sodium with ½ grain (0.06 gm.) of phenobarbital, 15 grains (1 gm.) sodium bromide, or 0.1 gm. of mesantoin are usually required in the more resistant cases.

Patients who are subject to petit mal seizures along with grand mal or psychomotor seizures should receive tridione plus phenytoin sodium, phenobarbital or mesantoin.

Status Epilepticus: Patients who are subject to seizures may have attacks so frequently that they do not recover from the coma produced by one attack before the next attack supervenes. The patient remains in coma for 12 to 24 hours during which time there may be many convulsive seizures. The attacks may cease spontaneously and the patient recover consciousness after a period of 24 to 48 hours, or death may occur as the result of the repeated attacks. The likelihood of the latter eventuality is so great that vigorous therapeutic methods aimed at terminating the seizures are justified. Good results in regard to termination of the attacks can sometimes be obtained by anesthetizing the patient with one of the volatile anesthetics such as chloroform or ether. Termination of the seizures is more certain with the injection of sodium phenobarbital or paraldehyde intravenously with less risk of pulmonary complications. It is important that a large dose be given at the first injection because best results are obtained when the full amount is given in one, rather than in divided doses. For status epilepticus in adults 0.4 to 0.8 gram (6 to 12 grains) of sodium phenobarbital dissolved in distilled water should be injected intravenously or 3 to 6 cc. of paraldehyde injected intravenously. The dosage for children should be from 0.2 to 0.4 gram (3 to 6 grains) of sodium phenobarbital or 2 to 4 cc. of paraldehyde according to the size of the child.

Summary

The treatment of patients with convulsive seizures requires a complete study of the patient in order to determine what factors are of

importance in regard to the occurrence of the seizures. All abnormalities should be corrected if possible. Surgical removal of cerebral lesions is of value in a small percentage of the cases. Psychotherapy, regulation of hygiene and schooling or a gainful occupation are necessary in all cases. The success in the treatment of the majority of the cases, however, depends upon the ability of the physician to properly use the anticonvulsant drugs which are available. The treatment can not be standardized. All of the standard drugs should be tried. If one is not successful when used alone, it should be administered in combination with one or more of the other drugs. The most common cause of failure is the administration of inadequate dosages.

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THE PRESENT STATUS OF LIVER FUNCTION TESTS INCLUDING OBSERVATIONS ON THE NEWER FLOCCULATION PROCEDURES*†

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THE liver is an organ with many functions and a large number of tests have been devised to evaluate clinically these divers functions. A classification of the tests based on the various physiological functions of the liver described by Greene and Bruger in 1943[1] has been revised and brought up to date. This classification is presented in Table I; the more important tests according to the concept of the present authors are capitalized.

In the following pages, a brief resumé of the present status of some of these liver function tests will be presented. In the strict sense of the term, the determination of serum bilirubin or of urine urobilinogen is not a test for evaluating the functional capacity of the liver but these laboratory procedures are included in this discussion because of their importance in the diagnosis of liver and biliary tract disease.

Bilirubin in the Serum: The most accurate index of jaundice is the quantitative measurement of bilirubin in the serum. Normal values using the method of Thannhauser and Andersen[2] (or any of its modifications) do not exceed 1.0 mg. per cent. The method of Malloy and Evelyn[3] utilizing a photo-electric colorimeter has a normal range not exceeding 0.8 mg. per cent. Recently, Ducci and Watson[4] recommended the determination of the prompt direct reacting bilirubin; normal values are not in excess of 0.2 mg. per cent.

Bilirubin in the Urine: During an epidemic of infectious hepatitis

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in the recent World War, Gellis and Stokes[5] utilized a modified methylene blue test to detect bilirubin in the urine. They found this procedure of value in the pre-icteric stage of infectious hepatitis. The test is simple to perform and may be used as a screen in the early detection of hepatic involvement as a result of exposure to certain industrial poisons. The reliability of the methylene blue reaction, however, has been questioned[6] since the test is not specific for bilirubin; yellow urines from normal persons and yellow substances such as potassium dichromate or ferric chloride will produce a green color when added to methylene blue.[7] The Harrison spot test[8] and the barium strip modification[9] may also be used to detect bilirubinuria and, for the most part, these procedures have replaced the older tests such as those of Rosenbach, Gmelin, etc.

Icterus Index: This does not have the specificity of the quantitative bilirubin since it merely measures the intensity of yellow color in the serum as compared with an arbitrary standard. The standard most commonly used is the color of a 1:50,000 solution of potassium dichromate. Any substance that will impart yellow color to the serum will elevate the icterus index. Thus, increased amounts of carotene, nicotinic acid, riboflavin and traces of hemoglobin may give false readings. Lipemic sera are also difficult to read and give false values. As a rough clinical guide, the icterus index has its place, but in cases where the presence or absence of jaundice is in doubt, the quantitative serum bilirubin is the test of choice.

Van Den Bergh Reaction: This color reaction forms the basis for the determination of quantitative serum bilirubin. The indirect and direct van den Bergh reactions do not differentiate between hepatocellular and obstructive jaundice since both reactions are present in these disorders. In the differential diagnosis of jaundice, its main value lies in the detection of hemolytic icterus since in this type of jaundice only an indirect reaction is obtained.

The form in which bilirubin exists in the blood stream probably accounts for the type of response obtained. Bilirubinglobin derived from the breakdown of hemoglobin in the cells of the reticulo-endothelial system gives the indirect reaction whereas bilirubin resulting from the breakdown of bilirubinglobin in the parenchymal cells of the liver produces the direct reaction. It follows, therefore, that a direct van den Bergh reaction is obtained in both hepatocellular and ob-

TABLE I.—CLASSIFICATION OF LIVER FUNCTION TESTS

I. *The Excretory Functions of the Liver*

A. *Formation and Secretion of Bile*

1. Secretion of bile acids
   a. Concentration of bile acids in blood
2. Excretion of bile pigments
   a. Bilirubin
      (I) Concentration of bilirubin in bile
      (II) Retention of bilirubin in blood
         A. QUANTITATIVE SERUM BILIRUBIN
         B. ICTERUS INDEX
         C. VAN DEN BERGH
            (1) Bilirubinglobin (indirect reaction)
            (2) Bilirubin (direct reaction)
      (III) Excretion of Bilirubin in urine
         A. Rosenbach's nitric acid method
         B. Iodine ring test
         C. METHYLENE BLUE REACTION
         D. HARRISON SPOT TEST
         E. BARIUM CHLORIDE STRIP METHOD
   b. UROBILIN AND UROBILINOGEN IN URINE
   c. Porphyrin (the urinary/fecal coproporphyrin ratio)
3. Excretion of cholesterol (concentration of cholesterol in bile)
4. Excretion of inorganic salts
   a. Sodium Chloride
   b. Calcium
5. Excretion of water

B. *Excretory Loading Tests*

1. Bilirubin
   a. Bilirubin excretion test
2. Dyes
   a. Phenoltetrachlorphthalein excretion test
   b. Rose-Bengal excretion test
   c. Azorubin-S excretion test
   d. BROMSULPHALEIN EXCRETION TEST
   e. Phenoltetraiodophthalein excretion test

II.I *The Metabolic Functions of the Liver*

A. *Carbohydrate Metabolism*

1. Fasting blood sugar
2. GALACTOSE TOLERANCE TEST
   a. ORAL
   b. Intravenous
3. Dextrose tolerance test
4. Fructose tolerance test
5. Utilization of d-lactic acid

(Continued on next page)

TABLE I.—*Continued from preceding page*

B. *Protein Metabolism*

1. Concentration of amino-acids in blood
2. Concentration of urea in blood
3. Concentration of ammonia in blood
4. Concentration of uric acid in blood
5. Concentration of guanidine in blood
6. Tyrosine tolerance test
7. Methionine clearance test

C. *Fat Metabolism*

1. Concentration of cholesterol in blood
   a. TOTAL CHOLESTEROL
   b. CHOLESTEROL PARTITION (ESTER/TOTAL RATIO)
2. Fat tolerance tests
   a. Oral
   b. Intravenous

D. *Blood Forming Functions*

1. Erythrocytes (Macrocytic hyperchromic anemia)
2. Plasma proteins
   a. Fibrinogen
   b. Albumin and globulins
      (I) CONCENTRATION OF SERUM ALBUMIN
      (II) Takata-Ara reaction
      (III) Formol-gel reaction
      (IV) Weltmann coagulation band
      (V) Magnesium chloride test
      (VI) Colloidal gold reaction
      (VII) CEPHALIN-CHOLESTEROL FLOCCULATION
      (VIII) THYMOL TURBIDITY
      (IX) THYMOL FLOCCULATION
      (X) COLLOIDAL RED PRECIPITATION
      (XI) ZINC SULPHATE PRECIPITATION
   c. Prothrombin
      (I) PROTHROMBIN TIME
      (II) RESPONSE OF PROTHROMBIN TIME TO VITAMIN K ADMINISTRATION

E. *Detoxification Function*

1. HIPPURIC ACID SYNTHESIS
   a. ORAL
   b. INTRAVENOUS
2. Cincophen test

F. *Phosphatase Production*

1. CONCENTRATION OF SERUM ALKALINE PHOSPHATASE

structive jaundice since in either condition bilirubin is regurgitated into the blood stream. The positive indirect response obtained in these disorders is due to the normal amount of circulating bilirubinglobin. In hemolytic icterus, there is no regurgitation of bilirubin but an excess of bilirubinglobin is present in the blood, hence a strongly positive indirect reaction alone is obtained.

Urobilin and Urobilinogen in Urine: Urobilinogen is formed in the small intestine by bacterial reduction of bilirubin. Part is excreted in the feces while the remainder is re-absorbed into the blood stream. The major part of this fraction is returned to the liver for re-excretion, while the rest is eliminated by the kidneys. It follows, therefore, that bile must enter the small bowel for urobilinogen to be produced. In complete obstruction of the extrahepatic type, there is a persistent absence of urobilinogen in the urine. In incomplete extrahepatic obstruction, the urinary urobilinogen varies between very low to normal or slightly above normal values. In hepatocellular jaundice, the urinary urobilinogen is low or absent in the acute phase followed by a gradual increase even above normal during recovery. This late increase is due to the inability of the liver to re-excrete that portion of urobilinogen which is returned to it via the enterohepatic circulation. In jaundice due to cholangiolitis, the urine urobilinogen may be absent in the early stages; thereafter, the values tend to be within normal limits. In our hands, the modified 2-hour test as described by Watson[10] has proved to be a satisfactory procedure for the quantitative estimation of urobilinogen in the urine. It should be emphasized that serial determinations of urinary urobilinogen are frequently necessary to establish the presence or absence of bile in the gastro-intestinal tract.

Bromsulphalein Excretion: Several dyes have been used clinically to test the excretory functional capacity of the liver. Of these, bromsulphalein[11] has won the widest acceptance. It is a sensitive and safe procedure with the added advantage of simplicity. The dye is removed from the blood stream by the Kupffer cells of the liver and then excreted by the parenchymal cells into the bile. Five milligrams of dye per kilogram of body weight is the usual dose given. Specimens of blood are then taken at 30 minutes and 60 minutes as recommended by O'Leary, Greene and Rowntree,[12] or a single specimen at 45 minutes as recommended by Mateer.[13] Using the latter, a normal liver should clear all the dye in 45 minutes so that none remains in the

serum. Values up to 4 per cent retention are considered normal if a photo-electric colorimeter is used instead of the usual comparator block. This test, however, is of very limited value in the presence of jaundice.

Galactose Tolerance: This test, introduced by Bauer,[14] is the one of choice in estimating the carbohydrate function of the liver. Unlike glucose, it does not require insulin for its metabolism and so far as is known, has no renal threshold. It has the added advantage of being rapidly absorbed. In the oral test, 40 grams of galactose dissolved in 400 ml. of water are taken with the subject in a fasting state, the urine collected for 5 hours and a quantitative estimation for urine sugar done. Normally, no more than 3 grams of sugar are excreted in the 5 hour period. The presence of impaired renal function introduces considerable error.

The galactose tolerance test is positive in acute and chronic parenchymal disease of the liver. It may be used in the presence of jaundice as an aid in differentiating parenchymal and obstructive jaundice. Bensley[15] reported a positive oral galactose test in 474 cases of toxic and infectious jaundice; in 210 cases of obstructive jaundice, the test was positive in 19 per cent of cases.

Bassett, Althausen and Coltrin[16] have developed an intravenous galactose tolerance test. In our opinion, the simpler oral test provides as much information as can be derived from the more complicated intravenous procedure.

Total Cholesterol and Cholesterol Partition: The value for total cholesterol in the blood plasma of normal adults varies between 160 and 230 mg. per cent. The cholesterol esters vary between 60 and 120 mg. per cent. The ratio of the combined to the total cholesterol is relatively constant ranging between 40 and 52 per cent when the Bloor[17] or modified Bloor techniques are used.

Thannhauser and Schaber,[18] Epstein and Greenspan,[19] Hurxthal and Hunt,[20] Klein[21] and Greene, Hotz and Leahy[22] have shown that in the presence of severe parenchymal damage to the liver, the cholesterol esters in the blood were reduced or entirely absent. Moreover, these workers noted that a reduction of cholesterol esters was seen more frequently in parenchymatous than in obstructive jaundice, this decrease being a better indication of the severity of the damage than as an aid in differential diagnosis.

Elevation of the total plasma or serum cholesterol occurs in obstructive jaundice of both the intrahepatic (cholangiolitic) and extrahepatic types. In most instances, the total cholesterol remains unchanged in the parenchymal forms of jaundice.

Serum Albumin: Since albumin is formed exclusively in the liver, it is not surprising that the concentration of this protein fraction in the blood is decreased in certain forms of liver disease. The normal level of serum albumin is 4 to 5 gm. per 100 ml. Tumen and Bockus[23] found that a reduction in albumin was the most consistent alteration in the serum proteins in patients with chronic advanced liver disease. Lowering of the albumin-globulin ratio was not found to be as significant or as constant as the reduction in serum albumin. Post and Patek[24] noted a rise in serum albumin during clinical improvement in patients with cirrhosis of the liver. Persistently low or decreasing serum albumin concentration was a poor prognostic sign.

Flocculation Tests: The flocculation tests are not strictly liver function tests. These procedures determine qualitative and quantitative alterations of various fractions of the serum proteins which result from disturbed activity of the parenchymal cells of the liver.

Cephalin-Cholesterol Flocculation: This test was proposed by Hanger in 1938.[25] He showed that in patients with active parenchymal disease of the liver, the serum flocculated a cephalin-cholesterol emulsion. Since then, many studies have confirmed this. Positive cephalin-cholesterol flocculation has also been reported in new-born infants with jaundice,[26] in cases of hyperthyroidism,[27] malaria,[28] in catatonics and schizophrenics[29] and in many other clinical disorders.

It is believed that gamma globulin is the protein fraction that takes an active part in the flocculation of the cephalin-cholesterol emulsion.[30] Under normal circumstances, the serum albumin has the capacity to inhibit the flocculating action of gamma globulin. In cases of parenchymal liver disease, the serum albumin fraction has a decreased capacity to inhibit this flocculating action on the part of the gamma globulin.[31] Readings of 2+ to 4+ are considered abnormal.

Thymol Turbidity and Flocculation: In 1944, Maclagan[32] described a new test of liver dysfunction utilizing a saturated aqueous solution of thymol buffered with barbital and sodium barbital at a pH of 7.8. He obtained essentially the same results with this thymol solution as with colloidal gold solution; in favor of the former was the ease with

which the solution could be prepared. Subsequent work confirmed the value of the thymol turbidity test as an indicator of abnormal activity of the liver parenchyma. The mechanism, however, is unlike that of the cephalin-cholesterol flocculation test.[33,34] Kunkel and Hoagland[35] showed that the turbidity of the thymol solution produced by certain sera depended on the presence of lipids and some abnormal lipo-protein associated with the beta globulin fraction of the serum. The gamma globulin fraction of the serum also played a role in the reaction. The relative importance of the different components in the reaction varied with different sera. As a general rule, in acute hepatitis cephalin-cholesterol flocculation becomes positive earlier than the thymol turbidity test while the latter remains positive longer during convalescence. Due to this fact and in view of the different underlying mechanisms, the thymol turbidity and cephalin-cholesterol tests supplement rather than supplant each other.[36]

Methods for quantitatively determining the degree of thymol turbidity produced by sera using various photo-electric colorimeters have been reported.[37,38] However, the original method reported by Maclagan, utilizing the formazin standards devised by Kingsbury an associates,[39] provides a simple and clinically accurate method. By this procedure, normal readings are 0 to 4 units.

In his original description of the thymol turbidity test, Maclagan stated that when the test was positive, flocculation frequently occurred in the tubes allowed to stand overnight; he felt, however, that this was not an essential part of the test. Neefe[40] noted that thymol flocculation remained positive for some time after the thymol turbidity reaction had returned to the normal range. In addition to supplementing and possibly increasing the sensitivity of the thymol turbidity test, the presence of 2+to 4+ flocculation at 18 hours or its absence may aid in evaluating the significance of borderline or weakly positive thymol turbidity readings.

Colloidal Red Test: Ducci[41] has recently recommended this procedure in which a colloidal suspension of scarlet red is used. The test is performed in the same manner as Maclagan's colloidal gold reaction. It is a sensitive and simple procedure and appears to parallel the cephalin-cholesterol flocculation and thymol turbidity tests. Positive flocculations probably depend on alterations in the serum gamma globulin fraction.

Zinc Sulphate Precipitations: Kunkel[42] reported that when serum with abnormally high gamma globulin concentration was diluted with a solution containing a small amount of zinc sulphate, a turbid precipitate formed and the optical density of the suspension was proportional to the concentration of gamma globulin. Normal serum did not flocculate for at least 12 hours while serum from patients with even slight elevation of gamma globulin fraction usually flocculated within 4 hours. While the test was not specific and was positive in cases having hypergammaglobulinemia from causes other than liver disease, it was found useful in determining the alterations in gamma globulin during the course of infectious hepatitis. Kunkel stated that the test was of particular value in detecting persistent liver disease following infectious hepatitis. In a group of 41 patients with cirrhosis of the liver, the reaction was positive in each case.

Prothrombin Time: The production of prothrombin by the liver is dependent upon ingested vitamin K. Bile salts are necessary for the absorption of vitamin K from the small bowel. In liver disease, the ability of the parenchymal cells to produce prothrombin may be impaired thus resulting in a disturbance in blood coagulation. Various methods are available for determining prothrombin time; in our hands, the Link-Shapiro[43,44,45] modification of the Quick method has proved most satisfactory, With this procedure the normal prothrombin time is 14 to 16 seconds for the undiluted plasma and 36 to 45 seconds for diluted (1:8) plasma.*

The response of the prothrombin time to the parenteral administration of synthetic vitamin K often offers aid in differentiating between hepatogenous and extrahepatic obstructive jaundice.[46] In the early stages of the latter, there is no impairment on the part of the liver cell to produce prothrombin. The increased prothrombin time is due to the impaired absorption of vitamin K resulting from a total absence or diminution of bile salts entering the duodenum. Thus, the parenteral administration of vitamin K leads to the restoration of normal prothrombin values in over 85 per cent of such cases. In patients where the jaundice is due to damage of the parenchymal liver cells, this restoration does not occur following the injection of vitamin K.

Hippuric Acid Synthesis: Hippuric acid synthesis in man occurs in the liver by the union of the benzoate radical with glycine. When 6

* These values vary with the type of thromboplastin used. In this institution, thromboplastin manufactured by the Maltine Company, New York, is employed.

gm. of sodium benzoate is given orally, the normal liver can produce 2.5 to 3 gm. of hippuric acid within 4 hours.[47] The excretion of less than 2.5 gm. of hippuric acid in the urine in 4 hours signifies liver damage providing, of course, that renal function is not impaired.

The test may be made more sensitive by giving the sodium benzoate intravenously.[48] In this instance 1.77 gm. of sodium benzoate in 20 ml. sterile water is slowly injected intravenously. One hour later, the patient voids and the hippuric acid concentration in the urine is determined. The normal range is 0.7 to 0.95 gm. of hippuric acid.

Hippuric acid synthesis by the liver is reduced in acute and chronic parenchymal disease and in metastatic carcinoma of the liver. It is normal in obstructive jaundice provided no secondary biliary cirrhosis has resulted from longstanding obstruction.

Serum Alkaline Phosphatase: Alkaline phosphatase is an enzyme concerned with several metabolic processes. Whether the liver produces alkaline phosphatase has not been definitely established; however, this enzyme is present in the bile. In cases of obstructive jaundice (intrahepatic and extrahepatic), the serum alkaline phosphatase is elevated. Normal values for adults by the Bodansky[49,50] method range from 4 to 6 units per 100 ml. of serum. An alkaline phosphatase above 13 units in an adult with jaundice is indicative of an obstructive lesion. It must be remembered that many other clinical entities may cause an elevation of the serum alkaline phosphatase, e.g., hyperparathyroidism, Paget's disease of bone, metastatic carcinoma to bone, etc. When properly evaluated, however, the serum alkaline phosphatase is a definite aid in the differential diagnosis of jaundice.

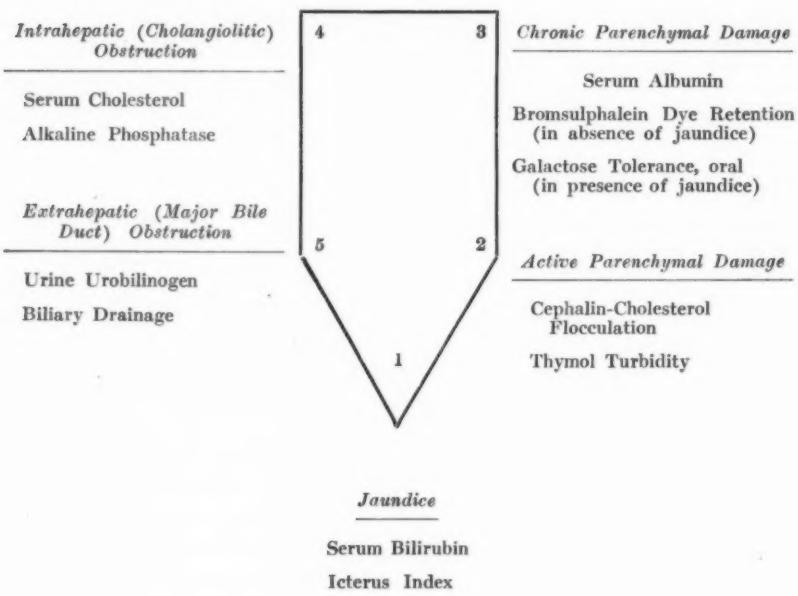
Discussion

The clinician is frequently overwhelmed by the large number of tests available for the estimation of liver function. A perusal of Table I exemplifies this state of affairs. For this reason, there is presented in Figure 1 a simple schema (modified after Greene[51]) coördinating the more important tests for estimating the functional capacity of the liver and for differentiating the various types of jaundice.

In this figure, liver and/or billiary tract disease is analyzed according to the presence or absence of five factors, namely, jaundice, active (acute) parenchymal damage, chronic parenchymal damage, intrahepatic obstruction and extrahepatic obstruction. The present authors

FIGURE 1.

SCHEMA FOR COORDINATION OF LIVER FUNCTION TESTS
(MODIFIED AFTER GREENE)



Types of Liver Disease	*Alteration in Pattern*
Hemolytic Icterus	1
Acute Hepatitis	1, 2
Cirrhosis of Liver with Jaundice	1, 2, 3
Cirrhosis of Liver without Jaundice	2, 3
Cholangiolitic Obstruction	1, 2, 4
Common Duct Obstruction	1, 4, 5
Common Duct Obstruction with Biliary Cirrhosis	1, 2, 3, 4, 5
Carcinoma of Liver (Primary or Secondary)	Irregular

have grouped these factors around a porphyrin ring because of the five points available in this configuration (peculiarly enough, the basic structure of bilirubin is a porphyrin ring). Thus, the presence of jaundice may be revealed by an elevation of the serum bilirubin and icterus index, active parenchymal damage by positive cephalin-cholesterol flocculation and thymol turbidity tests, chronic parenchymal damage by decreased serum albumin, by bromsulphalein dye retention in the absence of jaundice and by an abnormal galactose tolerance test in the presence of jaundice, intrahepatic (cholangiolitic) obstruction by an elevated serum cholesterol and alkaline phosphatase and finally, extrahepatic (major bile duct) obstruction by the persistent absence of urobilinogen in the urine and of bile in duodenal drainage. Below the figure, various types of liver disease are tabulated and the associated alterations in the pattern are indicated. Thus, in hemolytic icterus, there is jaundice as revealed by a high serum bilirubin and icterus index but no disturbances in the other four factors. In cirrhosis of the liver without jaundice only factors 2 and 3 are abnormal, namely, evidence of active parenchymal damage (positive flocculation tests) and chronic parenchymal damage (decreased serum albumin and bromsulphalein dye retention) and so on.

This schema is by no means complete or infallible. It is intended as a framework to which may be added other liver function tests depending upon the particular desires or experiences of the clinician. It possesses, however, simplicity and not infrequently affords diagnostic aid by the utilization of relatively few laboratory procedures.

Under the heading of flocculation tests, a number of procedures have been described which attempt to assay aberrations of the serum protein fractions resulting from disease of the parenchymal cells of the liver. In Table II the results obtained in five patients chosen from a large series[52] are detailed. Thus, in Case 2, a patient with infectious hepatitis, it may be noted that each of the flocculation or precipitation tests is abnormal. Case 3 is an example of the results obtained in early obstructive jaundice; each of the flocculation tests remains normal even in the presence of marked hyperbilirubinemia. When secondary biliary cirrhosis supervenes during the course of obstructive jaundice, the flocculation procedures become abnormal as may be noted in Case 4. The findings in active portal cirrhosis are shown by the results obtained in Case 5; hepatic damage is revealed by abnormal flocculation tests

TABLE II. COMPARISON OF SEVERAL FLOCCULATION TESTS IN PATIENTS WITH HEPATIC DISEASE

Case	Diagnosis	Icterus Index units	Cephalin-Cholesterol Flocculation 24 hour reading	Thymol		Colloidal Red Flocculation	Zinc Sulphate Precipitation				
				Turbidity units	Flocculation		1	2	3 hours	4	5
	NORMAL RANGE	4—6	0—+	0—4	0—+	0—2	0	0	0	0	0
1	Normal	6	±	1	0	0	0	0	0	0	0
2	Infectious Hepatitis	176	++++	12	++++	5	++	+++	++++	++++	++++
3	Obstructive Jaundice Due to Carcinoma of the Head of the Pancreas	121	0	1	0	0	0	0	0	0	0
4	Congenital Atresia of the Common Bile Duct with Secondary Biliary Cirrhosis	140	++	5	+++	4	0	±	+	++	+++
5	Portal Cirrhosis	24	++	5	+	5	+++	++++	++++	++++	++++
6	Familial Hepatic Dysfunction	12	+++	2	++	3	0	+	+	++	+++

throughout. In Case 6, a slight dissociation between the various procedures is demonstrated. In this patient with familial hepatic dysfunction, the thymol turbidity test alone remains normal; it may be assumed that the serum lipoproteins were not affected by the underlying hepatic dysfunction.

It appears worthwhile to stress again here that these simple though important laboratory procedures supplement each other. When these flocculation tests are carried out simultaneously, a better panorama of the alteration in the serum protein fractions is envisaged than can possibly be obtained by the performance of any single procedure alone.

Summary

1. A review and discussion of the practical liver function tests available to-day are presented.

2. The newer flocculation tests are described and compared.

3. A simple schema is presented coördinating several liver function tests as an aid in the diagnosis of diseases of the liver and biliary tract.

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REVIEW OF STUDIES ON BLOOD SUGAR*

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DR. MOSENTHAL and Members of the New York Diabetes Association, I thank you for your words of encouragement. This title, of course, permits a good deal of latitude to the lecturer, and what applies to the lecturer will also apply to the discussers who are to follow.

By way of introduction I should like to quote von Noorden's views on Diabetes Mellitus, as published at the beginning of this century in Volume III of his "Metabolism and Practical Medicine."[1]

"So long as we know no more about the nature of the diabetic process than we do at present we must, in common with former generations, define diabetes mellitus in terms of its most important clinical symptom—as a chronic disease in which grape-sugar is excreted in the urine. This definition however needs certain limitations, of which the following may be mentioned:

1. The quantity of sugar must be demonstrable by the ordinary clinical tests. The question whether normal urine contains traces of grape-sugar, to be detected only by the most delicate methods, may be left for the moment.

2. The grape-sugar must occur in the urine when the carbohydrate in the food is not more than that in ordinary human diet, or when the carbohydrate food is reduced in quantity or even stopped altogether. Cases where glycosuria only occurs after partaking of unusually large quantities of carbohydrate can scarcely be regarded as diabetes mellitus in a clinical sense.

3. The tendency to glycorusia must be persistent—that is to say, it must last at least some weeks or months. There are many conditions of ill-health in which a temporary disposition to glycosuria occurs. Such are not called diabetes mellitus, although there is considerable evidence that in both cases the glycosuria has the same fundamental

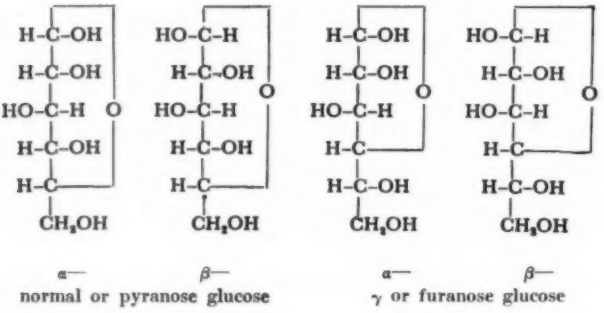
* Presented March 19, 1948 at an Open Meeting of the New York Diabetes Association at The New York Academy of Medicine.

cause (see anomalies of pancreatic function)."

I would draw attention to one point only, namely, the stress given to the estimation of sugar in the urine. Since that time emphasis has shifted from the estimation of sugar in urine to that in blood. I should now like to quote from Dr. Best's account of the "Discovery of Insulin" as narrated to the American Diabetes Association at the 25th Anniversary celebrations of its discovery. "It is obvious to all of you that we had many advantages over our predecessors in the search for the antidiabetic hormone. Certainly one of the greatest—probably the most important of all—was the availability of a good method for the estimation of sugar in small amounts of blood." It is with good methods for the estimation of sugar in small amounts of blood, and some results drawn from them that we are now concerned. No other constituent of the tissues is tested for as often as sugar. Yet no one has seen sugar from blood, for it has never been isolated as such. In 1892 Pickard is reported to have prepared from blood an osazone, which appeared to be identical with glucosazone. It would not seem to be a difficult matter to obtain a crystalline preparation of the sugar of the blood in reasonably quantitative yield. As far as I am aware it has not however been done. In spite of the lack of this direct and most satisfying of all proofs, there is a great deal of evidence supporting our general belief that the sugar of the blood is an equilibrium mixture of α – and β – glucose of the normal or pyranose type. In the fasting state we may say that it and it alone, in free solution, constitutes the uncombined sugar of the blood. In the cells there is a very small quantity of sugar phosphates; the amounts are always small. There is also present in blood plasma a relatively large amount of sugar of one kind and another, in the form of polysaccharides, conjugated with, and an integral part of the various globulin proteins. With such sugar constituents we are not concerned, though this combined sugar has been the subject of many scientific treatises. What we are concerned with is the freely diffusible reducing sugar of the blood, which under some conditions remains surprisingly constant in amount and yet at other times will vary in amount rapidly and widely.

Glucose is a reducing sugar and since it contains within its molecule asymmetric carbon atoms it is optically active; that is, its solutions rotate the plane of polarized light. At one time because the reduction value and the optical rotation value of a blood filtrate did not show

FIGURE 1



corresponding agreement, and because the optical rotation was lower than the equivalent reduction value, if the material being estimated was normal glucose it was supposed that some of the sugar existed in another form, α – and β –, γ – glucose. As can be seen in Figure 1, the α – and β – forms of γ glucose are represented as having a smaller ring structure than the corresponding forms of normal or pyranose glucose. This γ glucose has a lower specific rotation, and will reduce Fehling's solution rapidly even at room temperature and is therefore sometimes referred to as "reactive glucose." It was therefore seized on by some as the biologically active form of glucose. We now know, that the earlier evidence for such a reactive form of glucose was not valid because the various biological extracts, that of blood included, which were examined contained other substances besides sugar possessing both reducing and optical properties. There is, of course, nothing mysterious about this reactive form of the simple sugars. It is the reactive form of fructose (or laevulose) that is present in ordinary cane sugar, a fact that foiled chemists for years in their efforts to synthesize cane sugar. Fructose phosphates, to which reference will be made later, also have this smaller ring structure. There is no evidence, nor is there any need at present, to suppose that glucose is metabolized via "reactive" or γ glucose. It is now realized that the relatively inert glucose molecule becomes more active metabolically when esterified with phosphoric acid.

There are a variety of methods for the estimation of glucose in blood. The blood is first deproteinized, by one of a number of protein precipitants. The popular methods on this continent depend upon the

estimation of the sugar in the filtrate by heating under specified conditions with a copper solution. These copper solutions might all be regarded as modifications, some of them very considerable modifications, of the well known and prototype solution of Fehling, which will be celebrating its centennial next year.

Copper sulphate is a common constituent, but the other ingredients vary in identity and in amount. In general it might be said that those who have designed such modifications have had these objects in mind: firstly, a solution which will give the greatest reduction per unit of glucose, and secondly, the smallest reduction for those other constituents of blood filtrates which are also reducing agents. There are just two other points which should be mentioned here: firstly, with these various copper reagents and indeed with most other reagents used for the estimation of blood sugar there is no theoretical amount of reduction which should be made per unit of glucose present, i.e., one cannot calculate the amount of cuprous oxide formed by a known amount of glucose. This is so because the end aldehyde group of the sugar is not the only reducing grouping, since in the alkaline solution the glucose molecule split at a number of points and some of these split products are themselves reducing. It is therefore necessary to bear in mind the empirical nature of these estimations and the prime necessity of carrying out each estimation under the prescribed conditions. There should be strict comparison with the behavior of standard solutions of glucose of comparable concentration treated in precisely the same way. Some methods are not very reliable when glucose is present in low concentration. Secondly, a copper reagent designed by one investigator will not necessarily give the same reducing value with all kinds of blood filtrate. That is to say, different deproteinizing reagents give precipitates which carry down with them varying quantities of non-sugar reducing substances. The copper reagents purporting to give free sugar values should therefore be used with the particular kind of blood filtrate for which they were designed.

It is sometimes argued (sometimes, one suspects, just for the sake of argument!) that these so-called "true glucose" methods for blood, claim to be so because they give lower values than the older methods. This attitude gives rise to the expression "the so-called true blood sugar concentration. . . ." The imputation is that the lower the reduction value the more likely the designer or analyst is to call his method one for the

TABLE I. HIGH VALUES FOR NONGLUCOSE REDUCING SUBSTANCES IN A SUGAR TOLERANCE TEST

Timing	*Venous Blood Sugar, Mg. per 100 cc., True Blood Sugar*	*Venous Blood Sugar, Mg. per 100 cc., Folin-Wu Method*	*Nonglucose Reducing Substances, Mg. per 100 cc.*
Fasting	87	138	51
100 Gm. glucose by mouth			
½ hour later	102	176	74
1 hour later	109	178	69
2 hours later	90	168	78

According to the true venous blood sugar this is a normal curve, while the Folin-Wu method indicates a diminished sugar tolerance. This is an example of the hazard of an erroneous interpretation of blood sugars when the analyses include nonfermentable substances, whereas the correct diagnosis is revealed by the true blood sugar determinations.

Male (S. B. G.) age 44. Glycosuria found on Life Insurance examination 7-8 years ago; no glycosuria since that time. [Mosenthal and Barry (1946)]

estimation of true blood sugar, and the critic suspects that something has happened to the sugar, either it is lost in the protein precipitate or there is something in the filtrate or the sugar reagent which prevents the full reducing activity of the glucose. This is not necessarily so. The criteria which should be obeyed were those laid down very clearly by Benedict (1928).[2] Our techniques have improved but the criteria are essentially unchanged. The blood filtrate, after rapid fermentation with a relatively large amount of well-washed yeast, should give very little or no reduction with the reagent. The reduction, that is the glucose value obtained with the unfermented filtrate should not be less than the value obtained as the difference between total reduction before fermentation and residual reduction after fermentation by another standardized method. And lastly, the glucose added in known amount to the fermented filtrate should give the calculated reduction.

The titrimetric copper methods have been modified so that they may be turned into colorimetric procedures; as for example that of Nelson (1944)[3] and of Somogyi (1945).[4] In the clinical laboratory one method may be more convenient than another. But surely every effort should be made to use a method in which non-sugar reducing substances

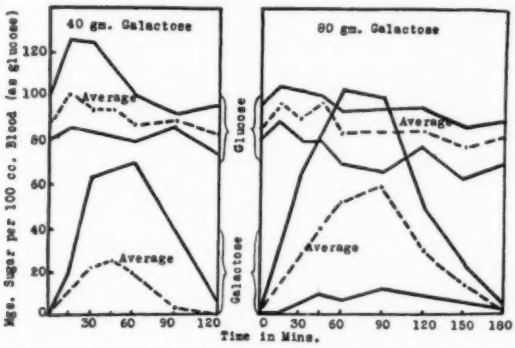


FIG. 2. Maximal, average, and minimal blood sugars after the ingestion of 40 gm. of galactose and 80 gm. of galactose. Average urine galactose after 40 gm. = 500 mg. Average urine galactose after 80 gm. = 2650 mg. [Harding and Grant (1933]

play little or no part. It is claimed with other methods in current use that while these non-sugar reducing substances give a substantial figure when calculated in terms of glucose, this value is relatively constant in different individuals in the absence of renal disease and that there is little variation in the same individual during the course of a glucose tolerance test. In the experiences of some, these assumptions are not justifiable, e.g. in Table I are shown results obtained by Mosenthal and Barry (1946).[5] Some clinicians state that it does not matter what method is used as long as they are told the normal limits as obtained by these methods. But I do not think this is a sound argument. If the non-sugar reducing substances vary from individual to individual and certainly if there is variation in the same individual over the course of the glucose tolerance curve, then surely a method giving true sugar values would be more reliable. It would be especially useful in cases of mild diabetes.

Mention might also be made here to the extensive work done on the "true sugar" of urine. The preliminary treatment of urine before quantitative testing for glucose was well explored by Harding and his associates (see Nicholson and Archibald[6]).

If galactose or fructose is fed alone, as is glucose in performing the oral glucose tolerance curve, there is a substantial increase in the sugar concentration of the blood.[7,8,9] With galactose the increased concentra-

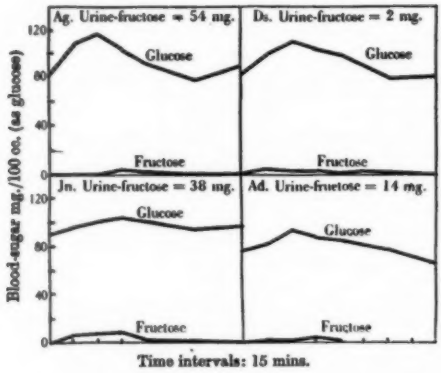


FIG. 3. Showing glucose and fructose in cutaneous blood after oral administration of 50 g. fructose. [Harding, Nicholson and Armstrong (1933)]

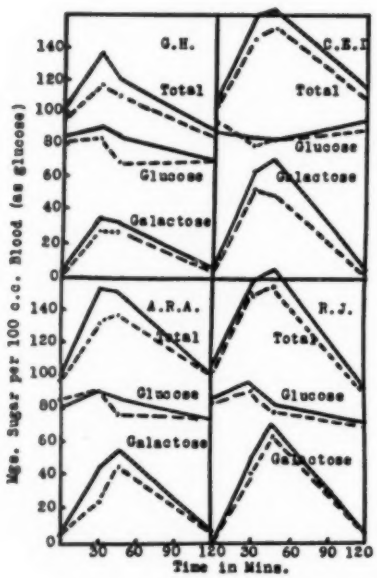


FIG. 4. Arterial-venous differences in blood sugars after the ingestion of 40 gm. of galactose. The solid line represents arterial blood; the broken line, venous blood. [Harding and Grant (1933)]

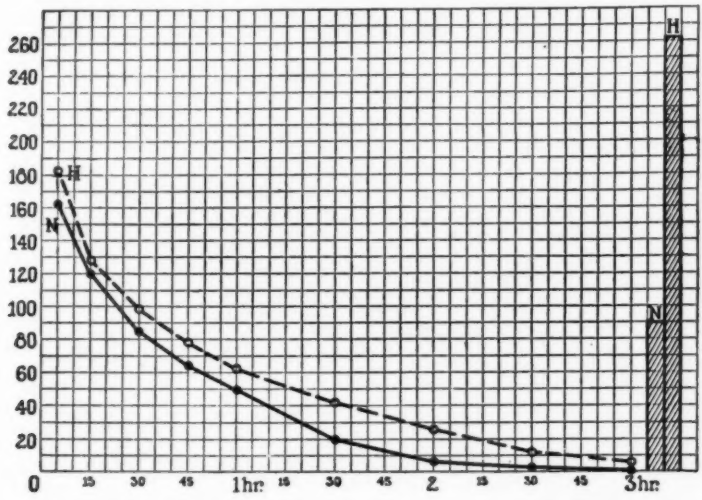


FIG. 5. Curves showing the clearance of galactose from the blood following the intravenous injection of 500 mgm. of galactose for each kilogram of body weight in the normal, *N*, and hepatectomized, *H*, dog. The curves are expressed in milligrams of galactose for each 100 cc. blood. The rectangles indicate the total amount of galactose excreted in the urine; shown as milligrams for each kilogram of body weight so that 500 would represent complete recovery of galactose. [Bollman, Mann and Power (1935)]

tion of blood sugar is largely due to the presence of galactose, and the maximum blood galactose concentration increases when increasing amounts of galactose are fed (Fig. 2). When fructose is absorbed the increase in blood sugar is largely due to an increase in glucose, the concentration of fructose being very small (Fig. 3). In the absorption of these hexoses from the small intestine it is supposed that all three are phosphorylated, and passed into the blood as free hexoses, with possibly some small amount of conversion of fructose and galactose to glucose during the absorption process.

The rate at which the galactose is removed from the blood is generally regarded as an index of the efficiency of the liver; at least as far as carbohydrate function is concerned. Galactose is of little value in the prevention of hypoglycemia in the dog following removal of its liver (Bollman, Mann and Power, 1935[10]). But there can be no doubt that galactose is used by tissues other than the liver (Fig. 4). Further when galactose is injected into a liverless dog the rate of removal of the galac-

tose is much the same as before removal of the liver (Fig. 5). There is a greater excretion of galactose in the urine of the liverless animal.

Recent work has shown that the kidneys can contribute to the maintenance of the concentration of blood sugar; while the liver must not therefore be regarded as the sole source of the blood sugar, it is easily the prime source, in quantity. The approximate amount produced under resting conditions may be gauged from studies on hepatectomised animals. The liverless dog requires about ¼ g./K./hr. to maintain a normal blood sugar level. For a variety of reasons the amount of sugar secreted by the liver ordinarily may be appreciably less than this. I should like to comment upon one piece of evidence only. It was shown that the oxygen consumption of the liverless dog is the same as before removal of the liver (Mann and Boothby, 1928[11]), and substantially the same results were obtained by Drury and McMaster[12] with the liverless rabbit. Bearing in mind the considerable metabolic activity of the liver, it is not easy to account for this lack of a decrease in oxygen consumption. It is possible that after the operation there is increased tone in the muscles: that is, the liverless animal is not really in the basal state. One is therefore led to the conclusion that the rate of usage of sugar by the peripheral tissues of the liverless animal is higher than that ordinarily provided by the liver in the resting animal. Crandall and Cherry by means of London cannulae suggest that the amount may be about 180 mgm./K./hr. for the normal dog.[13]

There can be no doubt that in the disposal of sugars absorbed from the small intestine or injected parenterally the liver plays an important part. The shape of the glucose tolerance curve and for that matter the fructose or the galactose tolerance curves are partly determined by the activity of the liver. It comes as something of a surprise to learn that the glucose and the galactose tolerance curves are so little altered in the liverless animal. Thus the intravenous injection of ½ g. per kilo body weight of galactose or of glucose in the liverless dog gives essentially the same die-away curves as in the intact animal. And yet it is well established clinically that the glucose tolerance curve is sometimes altered in liver disease; and that the excretion into the urine of galactose is appreciably increased in liver disease. Part of the explanation may well be that the liverless animal is not in the basal state: that is to say it is undoubtedly using more sugar (glucose or galactose) than would the peripheral tissues of the normal resting animal. This point is not

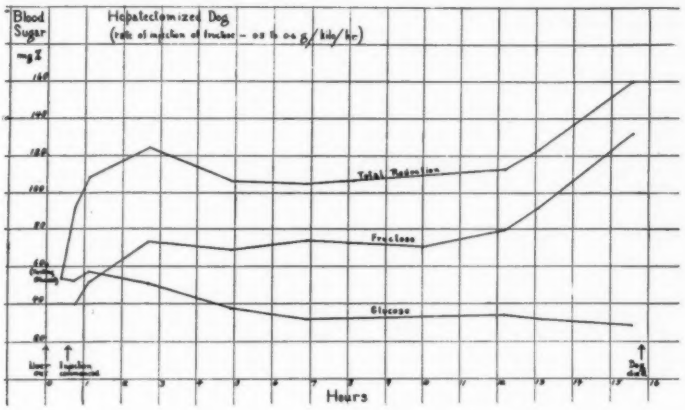


FIG. 6. [Griffiths and Waters (1936)]

generally appreciated. If it is borne in mind it will be seen that the interpretation of laboratory findings in clinical cases will be in better agreement with that of experimental work carried out on animals.

Fructose does prevent hypoglycemia in the liverless animal, but does not relieve the symptoms of hypoglycemia once established. We have found that while fructose will prevent the decrease in the blood glucose concentration of the liverless animal, there is no increase in the glucose concentration even when large doses of fructose are injected[14] (Fig. 6).

There is another interesting reaction with fructose which I think again concerns the liver and may well be part of the homeostatic mechanism concerned with sugar equilibria in that organ. The intravenous injection of fructose into a dog absorbing glucose from its intestine markedly decreases the concentration of glucose in the blood. That is to say, there is a very marked effect upon the glucose tolerance curve (Fig. 7). That this is not the result of a stimulation of the pancreas to secrete extra insulin, was shown by experiments on depancreatized dogs; for the same effect of fructose on the glucose tolerance curve could be demonstrated, provided insulin (exogenous in these animals) is present in the tissues (Fig. 8). A similar effect has been obtained on injecting a solution of sorbitol, instead of fructose.

A possible interpretation of this fructose effect may be based upon

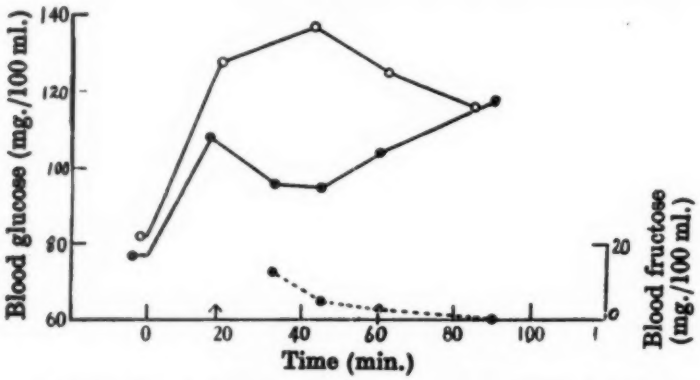


FIG. 7. The effect of fructose on the glucose tolerance curve of the normal dog.

o———o Glucose tolerance curve (control).

●———● Glucose tolerance curve after intravenous injection of fructose (0.5 g. per kg. body weight) at time marked with arrow.

●- - - -● Blood fructose values.

(Arrow indicates time of sugar injection. [Fletcher and Waters (1938)]

the recent investigations of the hexokinase and related enzyme systems. It will be seen (Fig. 9) that blood glucose is converted to glucose-6-phosphate by the hexokinase acting in conjunction with adenosine triphosphate. Glucose-6-phosphate is converted to glucose-1-phosphate and this latter compound through the mediation of phosphorlyase is converted to glycogen. Fructose can also be phosphorylated by the same hexokinase system and the fructose-6-phosphate so formed can be converted to glucose-6-phosphate. From a simple view of the mass action effects one would suppose that increasing the concentration of glucose-6-phosphate from fructose-6-phosphate would decrease the amount of glucose directly esterified to glucose-6-phosphate and therefore the blood glucose instead of decreasing would rather increase in concentration. Presumably some fructose-6-phosphate is converted to glucose-6-phosphate, but some passes directly to fructose-1:6-diphosphate. Fructose-1:6-diphosphate is converted by a series of changes into lactic acid and chemical energy is liberated, especially for the resynthesis of adenine triphosphate. It may be that the availability of this compound is the limiting factor in the synthesis of glucose-6-phosphate from blood glucose. In this manner one might account for the increased rate of removal of blood glucose following the administration

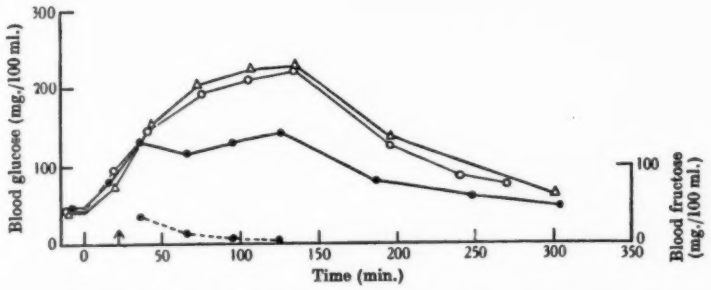


FIG. 8. The effect of fructose on the glucose tolerance curve of the depancreatized dog. Dog M. 16.3 kg. Insulin: daily 18 units, day before experiment 6 units a.m., 28 units p.m.

o——o Glucose tolerance curve (control).
Δ——Δ Glucose tolerance curve after injecting galactose (0.67 g. per kg. body weight).
●——● Glucose tolerance curve after injecting fructose (0.67 g. per kg. body weight).
●- - -● Blood fructose values after injecting fructose.
(Arrow indicates time of sugar injections.) [Fletcher and Waters (1938)]

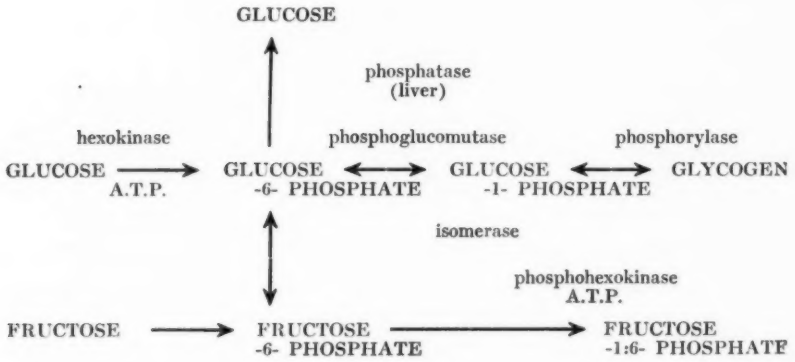


FIGURE 9

of fructose. Certainly there is evidence that fructose 1:6 diphosphate is formed, because there is an appreciable increase in blood lactic acid in animals receiving fructose.

This fructose test may have some application of clinical interest. While an injection of fructose into a normal fasting dog or into a fasting depancreatized dog with relatively low blood sugar following the administration of insulin produced no decrease in the blood glucose con-

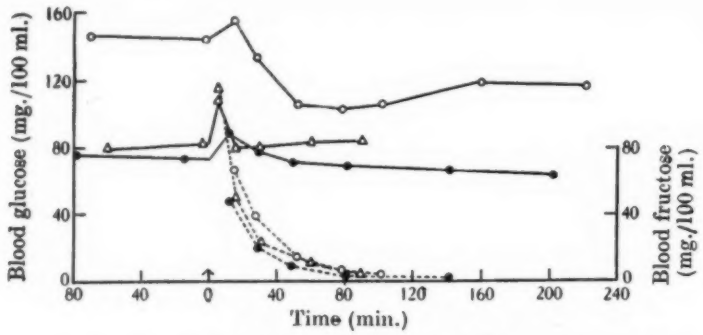


FIG. 10. The effect of fructose on the blood glucose concentration of the fasting normal dog and of the fasting depancreatized dog.

Δ——Δ Glucose } Fructose (0.5 g. per kg. body weight) injected into
Δ- - -Δ Fructose } normal dog.

o——o Glucose } Fructose (0.67 g. per kg. body weight) injected into
o- - -o Fructose } fasting depancreatized dog (receiving insulin) with high blood sugar.

●——● Glucose } Fructose (0.5 g. per kg. body weight) injected into
●- - -● Fructose } fasting depancreatized dog (receiving insulin) with low blood sugar.

(Arrow indicates time of sugar injections.) [Fletcher and Waters (1938)]

centration, it was possible on two occasions to demonstrate a decrease in blood glucose in the fasting animal. On these occasions the depancreatized dogs had received protamine zinc insulin the night before, as usual, but the following morning the blood sugar was appreciably above the normal fasting level; injection of fructose into these animals produced a significant decrease in the fasting blood glucose concentration (see Fig. 10). Thus an intravenous injection of fructose into a fasting diabetic patient may lower, temporarily, the high blood glucose, or there may be an immediate increase in the blood glucose. The former reaction might be interpreted as indicating that some insulin was still being supplied by the pancreas, while the latter response might indicate an absent or very small supply of endogenous insulin. This, of course, is speculation. Certainly no such experiments have been done.

This rather curious effect of fructose which can be demonstrated in dogs may provide an explanation for earlier reports that, for a short time at least, some diabetic patients were said to be able to assimilate added fructose better than a corresponding extra quantity of glucose, as judged by the resulting glycosuria. There may be an increased synthesis of glucose to liver glycogen.

It has already been pointed out that the best known reaction of insulin, namely its lowering effect on the blood sugar, was the reaction most useful, indeed it was the indispensable test, in its isolation. The next best known reaction of insulin is on the synthesis and storage of glycogen, both in muscles and in the liver; though the role of insulin in the storage of glycogen in the liver is on occasion equivocal. Some would point to the part played by the hyperglycaemic factor, of which we shall have more to say later. However, the deposition of glycogen was the first clue as to what happened to some at least of the blood sugar under the action of insulin. There were many who made the far-reaching interpretation from this sort of experimental finding, that before glucose could be metabolised it had to be synthesised to glycogen. The evidence for this view was always circumstantial and we now know, particularly through the isotopic studies of Stetten and his associates,[15] that the bulk of glucose is not transformed into glycogen. Glycogen is a storage form of glucose, and, provided there is ample blood sugar, insulin will cause increasingly large deposits of glycogen. More recently and again as a result of isotopic studies with deuterium, Stetten and Klein (1946),[16] supporting and extending earlier conclusions of Drury and others, have shown that insulin causes a rapid conversion of blood glucose to fat, presumably, in the liver. Thus fat can be regarded as another form of storage of blood glucose, and indeed much more of the blood glucose of an ordinary mixed diet is stored as fat, under the action of insulin, than is stored as glycogen. We may still regard insulin as indubitably playing the role of a storage hormone: converting blood glucose above a certain concentration to fat and glycogen for later use. This storage mechanism is radically interfered with in the absence of sufficient insulin. What other role or roles insulin plays is still being vigorously investigated; such for example as the effect of insulin on oxygen consumption—is the oxygen consumption increased or is oxidation shifted from one type of metabolite to another?

It will be recalled that Boxer and Stetten (1944)[17] showed that far more glycogen is formed from glucose in the previously fasting rat receiving a solution of glucose by mouth than is formed in the rat feeding in normal fashion on a high carbohydrate diet. They supposed that this difference was due to the fasting state. Now it is known that fasting tends to decrease the formation of glycogen (at least less glycogen is deposited in a rat receiving glucose previously fasted 48 hours than in

a rat fasted 24 hours.) Therefore one might suppose that the effect of fasting for 24 hours as in Stetten's experiments would be to decrease glycogen synthesis over what would occur in a well-fed rat. The explanation for the difference might reasonably be that in the case of rats fed on the high carbohydrate diet (starch) the blood sugar would not rise as high as in the rat fed glucose, and therefore less glucose would be stored as glycogen in the former animals. That is to say, the effect of insulin is to remove rather rapidly blood sugar in excess of a certain concentration, and the higher the glucose level above that concentration the more storage of glycogen will occur. Whether the much higher blood glucose concentration in rats receiving glucose only, results in an increased rate of secretion of insulin from the pancreas is still something that one might mention without being able to throw any more light on the problem. Other hormones we know play their part: a more satisfying description of what happens in this instance and in others will be obtained when further knowledge of the enzyme systems involved is elucidated and the way in which the various hormones influence these enzymic reactions.

A good deal of interest has been aroused amongst laboratory investigators recently about the hyperglycemic factor present in some preparations of insulin, both crystalline and amorphous. Whether the substance responsible for this effect is present as such in the pancreas or whether it is a product formed during the isolation of insulin has yet to be determined. If the former is the case, it would at least prove of continued interest to physiologists. The physiological significance has yet to be shown; so also has its clinical significance. It has been stated that only "novo" insulin, produced in Denmark, is consistently free of this hyperglycemic material.

Shipley and Humel (1945)[18] incubated rat liver slices in the animal's own serum and noted that the glycogenolysis which occurred was increased by the addition of insulin. They also found that the rate of accumulation of new carbohydrate was increased. These effects were obtained with liver slices, possessing a high initial glycogen content, from well-fed animals. Having in mind the experiments of Bouckaert and de Duve as described in their review (1947)[19] on the action of insulin, it was natural to suspect that this effect on rat liver glycogen which Shipley and Humel obtained might be attributed to the hyperglycaemic factor which Bouckaert and de Duve had shown to be

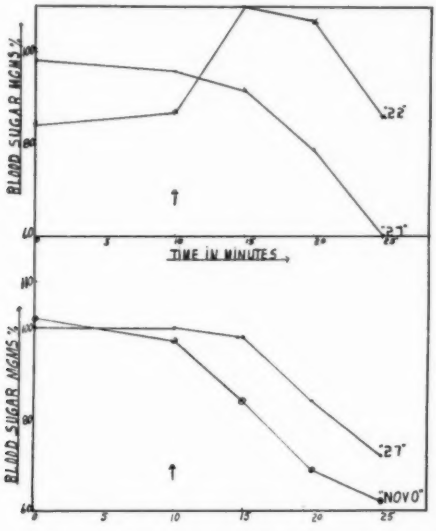


FIG. 11. Effect of insulin on fasting blood sugar. (Arrows indicate times of insulin injections.) [Balasubramanyam and Waters]

present in some insulin preparations. They were able, for example, to show that the fall in the blood sugar concentration of a rabbit injected with 30 units of insulin, free of the hyperglycaemic factor, could be prevented either by an infusion of glucose at the high rate of 1.4 g./k./hr. or by an infusion of about 20 u./k./hr. of another brand of regular insulin. I therefore decided, with Mr. G. Balasubramanyam of Madras, India, at present a postgraduate student in Dr. Best's laboratories, to investigate more closely this effect of insulin on the glycogen of rat liver slices. These experiments are still in progress; the conclusions we draw from them will therefore be somewhat speculative and will await confirmation by further tests which we are now carrying out. Our plan was to use two samples of crystalline insulin, of different potency but manufactured by the same pharmaceutical house. We are greatly indebted to Mr. A. H. Lacey of the Insulin Committee Laboratory at the University of Toronto for these two assayed samples of insulin. One sample assayed at 22 units per mgm. the other at 27 units per mgm. We presumed that the insulin of lower potency would con-

tain more of the hyperglycaemic factor. Solutions of these two preparations were therefore injected intravenously into fasting rabbits in amount equal to 1 unit per kilo body weight and it was soon apparent that the less potent insulin preparation did give a definite preliminary hyperglycaemia, while the more potent preparation gave only a slight hyperglycaemic effect. Indeed, it would be truer to say that, whereas a sample of "novo" insulin, of Danish origin, known to be free of hyperglycaemic material caused an immediate decrease in the blood sugar of the fasting rabbit, our more potent preparation—the one assaying at 27 units per mgm.—did not cause hyperglycaemia but rather showed a lag in its production of hypoglycaemia (Fig. 11).

The effect of the two insulin samples, which for convenience we have called insulin 22 and insulin 27, was also obtained on rat liver slices from well-fed animals. Three slices of liver, each of about 50 mgm. weight and about 0.3 mm. thickness, were placed in three Warburg vessels, each containing 1 cc. of the rat's serum. The first slice was the control. To the second slice 2 units of 27 insulin were added and to the third slice 2 units of 22 insulin. The vessels were gassed with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide, and incubated, with shaking, at 37°C. for 2 hours. In this way we have therefore followed out the general procedure of Shipley and Humel. We found, as they had, that the addition of insulin to the incubated liver slices caused an increased breakdown of glycogen and an increased accumulation of free sugar, over that found in the incubated slice, without added insulin. We have one additional observation to make, which we think may have considerable importance if our speculations can be substantiated in further experiments. In the vessels containing insulin 22 the glycogen concentration of the liver slice fell to about the same value as did the glycogen concentration of the slice in vessels receiving insulin 27, but the amount of free sugar which accumulated in the former vessels was appreciably more than in the latter vessels. And the amount of free sugar which formed in these vessels was considerably above that produced by the observed glycogenolysis. So far we have done no oxygen determinations, and it may be that insulin 22 inhibited the oxidation of free sugar. If we assume that this is not so, then we should like to put forward the tentative suggestion, that this hyperglycaemic factor present in both samples of insulin, but in greater amount in insulin 22, causes not only glycogenolysis but also

TABLE II. GLUCOSE CHANGES IN RAT LIVER SLICES IN VITRO. EFFECT OF INSULIN

Rat No.	Serum glucose initial mgm. %	Serum glucose after incubation per 100 mgm. of liver slice			Increase in glucose produced per 100 mgm. of liver slice	
		Control mgm. %	Insulin 27 mgm. %	Insulin 22 mgm. %	Insulin 27 mgm. %	Insulin 22 mgm. %
1.	121	422	515	674	93	152
2.	159	419	434	826	15	407
3.	153	320	395	603	75	282
4.	250	356	347	687	-9	331
5.	173	348	368	444	20	96

Rat No.	"Total glucose content" of liver slices, gm.%				Glucose produced by glucogenolysis per 100 mgm. liver slice	
	Initial	Control	Insulin 27	Insulin 22	Insulin 27 mgm. %	Insulin 22 mgm. %
1.	7.21	2.93	2.42	2.35	51	58
2.	7.33	2.40	1.54	1.538	86	86
3.	5.91	2.10	1.57	1.55	53	55
4.	12.56	4.38	4.36	4.18	2	20
5.	6.14	2.30	1.76	1.40	54	90

[Balasubramanyam and Waters]

new formation of sugar (gluconeogenesis) (Table II). If we are so rash as to presume this to be a physiological mechanism, then the lack of insulin effect on sugar production which Shipley and Humel obtained with liver slices from fasting rats might be attributed to the fact that gluconeogenesis was already proceeding at a rapid rate and the hyperglycaemic factor would not therefore be expected to make any further appreciable increase. Very recently, Sutherland and Cori (1948)[2c] have investigated the effect of addition of "Novo" insulin and of insulin preparations containing the hyperglycaemic factor, on rat liver slices incubated in various buffered phosphate solutions. They showed that insulin which had been inactivated, for example, with alkali, still exerted its glycogenolytic action but "Novo" insulin, which possessed no glycogenolytic action, when similarly treated with alkali, did not develop glycogenolytic properties. One wonders whether this might not be regarded as circumstantial evidence that the hyperglycaemic factor may exist as such in the pancreas. In the experiments of Suther-

land and Cori, of the glycogen that disappeared some 60 per cent might be accounted for in terms of the increase in free reducing sugar. There is no evidence in these experiments of any gluconeogenesis. But these workers did not incubate their slices in serum, with its supply of protein and other metabolites, but in inorganic phosphate solutions. This seems likely to be a fruitful field of experimentation.

There is a further point. You will have observed that we have been using insulin preparations of differing potency. The one at 22 units per mgm. is identical in potency with the international standard of insulin; the other is appreciably higher, namely 27 units per mgm. Whether this difference is wholly due to greater admixture of the less potent preparation with the hyperglycaemic material is not clear. But since the more potent preparation also contains some small amount of the hyperglycaemic factor, we may reasonably predict that insulin preparations assaying at a slightly higher figure than 27 units per mgm. may be obtained. I understand that "Novo" insulin assays at about 23 units per mgm.

In conclusion: it can be stated that methods for the estimation of sugar in blood have steadily improved. Greater reliability has been obtained without unduly increasing the complexity and tedium of the analysis. It is true that we have not got a chemical method specific for glucose: from the very nature of glucose that is understandable, for all methods rely upon its reducing properties. But with the use of various micro-organisms, such as yeast, proteus vulgaris and several others a high degree of specificity can be imposed on the relatively non-specific reduction procedures.

We realise that when we determine the concentration of the blood sugar we are really dealing with the equilibrium. Sugar is entering the blood at certain points and leaving it at others. Hormones we know influence these rates of production and removal; and not only hormones for we have seen that even the injection of another sugar, namely fructose, will bring about a profound effect on the equilibrium. Of the hormones we have made some reference to the part played by insulin. We have also dealt with, shall we say, a hormone presumptive or a hormone pretender—the hyperglycaemic factor. It is apparent that there are a multiplicity of factors governing the height of the blood sugar level—and yet how very useful it is to have accurate methods for its measurement.

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Discussions by

FREDERICK M. ALLEN, EDWARD S. DILLON, THOMAS H. MCGAVACK, and CHARLES H. BEST

continued on next page

DISCUSSIONS

FREDERICK M. ALLEN

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In trying to discuss Dr. Waters' very scholarly paper I first take the opportunity to inquire whether he or his colleagues can throw any light on a problem which was discussed in my book published in 1913, namely diabetic polyuria. Although it may vary in early mild cases and in some late cases after the kidney has developed a high threshold or perhaps organic impairment, this symptom is a typical feature of full-fledged diabetes. In contrast to the superficial assumption that glucose is a diuretic, I was struck by the fact that it is not a diuretic when given by mouth, subcutaneously or intraperitoneally. By these routes it is anti-diuretic; it diminishes urine; and only when given intravenously is it a diuretic. This rule holds also for animals which are depancreatized to a degree which does not cause diabetes but reduces the tolerance so that marked hyperglycemia can be produced more easily than in the normal.

Speculatively, I suggested that the glucose given by vein is a crystalloid, but when given otherwise it enters into some kind of linkage so that it behaves as a colloid. But chemists have failed to find even the loosest linkage or to sustain the alpha-beta-gamma distinction or any other difference between diabetic and non-diabetic blood sugar. On the other hand I overlooked the fact that glucose given by vein induces a marked hydremia, altogether different from the condition in diabetes. The failure of a speculative explanation should not distract attention from the observed fact. It may be argued that glucose in the intestine or in the tissues holds water by osmosis, or that glycogen is stored together with water in the normal organism while the diabetic fails to deposit glycogen. But the essential fact is that if adequate water is supplied, the administration of glucose orally, subcutaneously or intraperitoneally causes hyperglycemia and glycosuria accompanied by slight hydremia and marked oliguria. This is in contrast to the polyuria which should result from this degree of hydremia without the glucose, and it is opposite to the diabetic condition, in which hyperglycemia is not accompanied by hydremia and the glucose diuresis is so powerful that it can cause polyuria with desiccation of the tissues and concentration of the blood. The seemingly intermediate results in phlorizin or clinical renal glycosuria may deserve study. My impression also is that normal animals injected with sugars which are assimilated poorly or not at all, such as lactose, sucrose, galactose or pentoses fail to equal the diuresis of diabetes. Perhaps these phenomena would be explainable if I had been in position to utilize the recent methods of studying kidney function, but at least superficially the diabetic organism seems to differ from the normal in its handling of glucose not only in the general tissues but also in the kidneys.

On the practical side, I was the first to advocate treating diabetes according to the standard of normal blood sugar, and also the first to contradict the tradition of spontaneous progressiveness of the disease. The two ideas are connected. Also the prevention of progressiveness includes complications. After these many years, there is still need to defend the thesis that diabetes and all its accompaniments can be and should be controlled.

Theoretically, excessive blood sugar is an abnormality, and we should not risk people's lives on an unproved assumption that a chemical abnormality can continue for years without harm. Also diabetes is known to be not merely an over-production of sugar but a defective assimilation; therefore it appears irrational to claim benefit from a high sugar due to lack of assimilation when we have means of improving the assimilation so as to keep the sugar normal.

Hypothetically, the harm of prolonged hyperglycemia might be physical, chemical or metabolic. My previously mentioned book included experiments with long continued injections of cane sugar in cats. The damage to the nervous system, particularly in one animal which became obese, paretic and demented, could perhaps be attributed to the osmotic action of the sugar, and there might be interest in more prolonged and less intensive experiments with a non-assimilable sugar such as lactose with respect to possible arterial lesions. I tried unsuccessfully to break down the tolerance of non-diabetic animals with glucose, but with this method of simple glucose injections Lukens succeeded in producing hydropic degeneration of islands and permanent diabetes in cats. The traditional progressiveness of poorly controlled diabetes is thus clearly explained, and if the pancreatic islands are so sensitive to this clinical stimulus there is reason to suspect some effects on other organs. My own concept is that the metabolic disturbance extends deeper than the mere sugar molecule, probably altering the entire mixture of intermediary products and enzymes. Diabetes is a specific malnutrition. An elevated blood sugar level is chiefly important as the most delicate indicator of this malnutrition. If the malnutrition is long continued, functional and anatomic deterioration may naturally be expected in the blood vessels, nerves and other organs, without the need of incriminating fat or any other single substance as a primary cause. The distinction from undernutrition is illustrated by the pre-insulin experience of clearing up diabetic complications and improving strength by undernutrition or starvation. When either undernutrition or insulin corrects the specific malnutrition according to the most delicate tests, my personal testimony is that the progressiveness and the complications of diabetes remain absent, the degrees of damage which may be present on beginning treatment are arrested, and health is maintained indefinitely.

For testimony other than my own observations, I have had a few illustrative cases examined carefully as to the state of their heart, retina and leg arteries by specialists in those respective fields. The first of these patients is a 76-year-old man who has had diabetes more than 20 years and whom I have treated for 19 years. He has no subjective complaints, carries on his business as actively as ever, and runs two miles before breakfast every day when the weather is clear. The pulses in his legs and feet are normal. I am showing you an X-ray picture of his legs with no calcification of the arteries. His retina and his heart are reported thoroughly normal. Obviously this man merely happens to belong to a small class of persons who are remarkably well preserved at an advanced age, but the essential point is that his diabetes did not keep him from being well preserved.

I shall omit several similar examinations of patients above the age of 60 who are symptom-free and who show no organic or vascular changes beyond those normal for their years. They merely conform to the rule that persons who are normally controlled remain normal. Some other patients wander away for several years, taking plentiful insulin to maintain weight and comfort but eating carelessly so that sugar is high in blood and urine. They often return on account of retinitis, heart symptoms, gangrene, neuritic pains or other complications. Though some of these conditions are hopeless, control of the diabetes does frequently clear them up and it is always the most important feature of the treatment. Since I find the prevention of these troubles in normally controlled patients to be uniform, without any exceptions, I have been watching for accidental lesions. For example, a certain proportion of elderly persons should be subject to purely arteriosclerotic gangrene, without any diabetic factor. It can only be said that during the past 28 years even the slightest beginning of hypertension has been my indication for reducing or excluding salt in the diet. Also the valuable clinical-pathological study of Wilens, showing an inverse ratio between body weight and arteriosclerosis, implies that the sclerosis is at least to some extent controllable by diet; and the limitation of fat, total calories and body weight was my original principle of diabetic diet. Therefore it is conceivable that, instead of the

traditional excess of arteriosclerosis, there may be a reduction of incidence below that of the general population. At any rate I still can repeat my standing invitation to ophthalmologists and other consultants who see my patients, to point out any one of them who has followed treatment and who develops any diabetic complications.

The next slide shows the photograph of a 14-year-old girl who has had diabetes only 9 years. She is even better looking than her picture. Her eyes, heart and leg arteries are normal. None of her playmates has any happier life or can surpass her in any sport. She illustrates the smooth control of diabetes in a young child with 58 units of insulin daily. I show her partly to place on record a declaration that she will be normal 10 years and 20 and more years from now, and if she marries she can have normal pregnancies.

The final two slides show diabetic mothers and their young children. The first of these is a South American, whose diabetes began 19 years ago, at the age of 16, and I have treated her for 18 years, by occasional check-up visits to this country. Her first pregnancy under the care of local doctors ended in acidosis and abortion. She remained in New York during nearly the whole of her second pregnancy and it was entirely normal. She expects to come here for the same purpose again and I predict a similar result. The second patient became diabetic 22 years ago, at the age of 12 years, and I treated her for 21 years. After having a large ureteral stone removed through a lumbar incision, she went through a pregnancy which was normal except for large size of the baby. This tendency among diabetics is perhaps explainable as a nutritive effect of the insulin. Because of the handicap of distance for the first patient and the rather difficult fluctuating type of diabetes in the second, their records are not perfect and therefore perfect results are not theoretically to be expected. Prompt correction of irregularities, however, goes far toward preventing complications, both puerperal and non-puerperal. There is no question of the necessity of special treatments after pathological changes have occurred in the arteries and organs. But my impression is that when such changes are found in allegedly well managed cases, examination of the actual records will show hyperglycemia not slight or brief in character but of marked degree for long periods or habitually. My experience is that thorough control of the diabetes from an early stage prevents the specific lesions and eliminates the need for special treatments.

My radical position on the prevention of progressiveness and sequels in both diabetes and hypertension is referred chiefly to the judgment of posterity. This formerly solitary claim seems to be gaining more support from later writers, in proportion as they conform to the methods. But the enormous toll of disability and mortality justifies regret for the long wasted years without the experimental and clinical settlement of this problem which I had once planned.

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Edward S. Dillon

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Doctors Waters' paper shows us a sample of the tremendous amount of work which is going on in the field of carbohydrate metabolism resulting in knowledge which has been accumulating rapidly during the past 25 years. As is true in the case of all great discoveries, insulin has posed a great many more questions than it has answered.

I suppose that this audience is composed almost entirely of clinicians, like myself, and I suppose, too, that most of us have trouble in keeping up with all the work and discoveries of our good friends, the biochemists and physiologists. I remember from my own days in medical school, about 35 years ago, that my knowledge of glucose

metabolism was pretty much limited to the fact that one molecule of glucose was oxidized by six molecules of oxygen to produce six molecules of carbon dioxide and six of water. We spoke of the energy supplied to the body by the "carbohydrate fire," as though the oxidation of glucose to carbon dioxide and water was consummated in a single step. Little conception did we have then of the intermediary steps of glucose metabolism; of the anaerobic phase consisting of ten steps from glucose to pyruvic acid in which phosphorylation plays the leading role; of the aerobic phase below pyruvic acid, in which one molecule of pyruvic acid joins with one molecule of oxalacetic acid and goes in eight steps around the Krebs Cycle, the pyruvic acid molecule being entirely broken down and the oxalacetic acid molecule returning to its starting point, ready to take on another molecule of pyruvic acid. All this is a marvelous sequence of chemical reactions, most of them reversible, in which the body receives energy at the relatively low temperature of the body and in the quantities needed at the moment. How different all this is from a "carbohydrate fire"!

Practicing physicians come in touch with blood sugar estimations chiefly under two circumstances, first in the diagnosis of diabetes or hypoglycemia, and second, in guiding the treatment of patients with diabetes.

Frequently, sugar is found in the urine of a patient who has no symptoms and we are called upon to decide whether he has diabetes. The normal figures of the blood sugar tolerance test were established at a time when the blood sugar methods measured copper reducing substances other than glucose as well as glucose. As Doctor Waters has shown tonight, non-glucose reducing substances may be present in sufficient quantities to result in an apparently abnormal blood sugar tolerance test if the method employed measures other copper reducing substances as well as glucose.

The older blood sugar tolerance standards were done on venous blood. Now capillary blood is frequently used. The sugar content of capillary blood is often considerably higher than that of venous blood.

Perhaps we need a new evaluation of what are to be considered normal figures, using capillary blood and methods giving true glucose values. Fortunately, when the figures are borderline abnormal, the changes which occur with the lapse of time are more important than our opinions as to exactly what constitute normal figures.

The second use for blood sugars which we clinicians have is in connection with keeping our diabetic patients under good control. A single blood sugar determination, if very high or very low, will at once, point the direction our treatment should take. A single moderately elevated blood sugar, 200 for example, may be quite meaningless unless we take into consideration the chief factors which determine the 24 hour blood sugar pattern of this particular patient.

1. Time—When was this blood taken with reference to meals? When was it taken with reference to insulin injections?
2. Insulin—What kind of insulin and how much?
3. Kind of Patient—Is he one whose blood sugar level changes rapidly under the influence of insulin (insulin sensitive) or does his blood sugar level change slowly (insulin resistant)?

These factors must be given careful attention when one is evaluating the meaning of blood sugar reports, or else serious errors are likely to occur. The following are several examples:

(A) A patient is taking 50 units of protamine daily. Fasting blood sugar is 200. This is not satisfactory because in patients taking protamine insulin the blood sugar level is near its low point before breakfast. This patient needs more insulin.

(B) A patient is taking 50 units of protamine daily. Blood sugar taken 2 hours after breakfast is 200. This is probably satisfactory as the fasting blood sugar level was probably much lower before breakfast. Protamine insulin usually does not prevent sharp post-prandial peaks in the blood sugar level.

(C) A patient is taking 30 units of regular insulin before breakfast and 20 units of regular insulin before supper. Fasting sugar is 200. This

is probably satisfactory as this is likely to be the high point of the blood sugar level for the day. Regular insulin is likely to be used up shortly after midnight and the patients own endogenous insulin may be insufficient to keep the blood sugar level down during the rest of the night. The blood sugar may go up during the small hours of the night, even though no food is taken.

(D) A patient is taking 30 and 20 units of regular insulin as in (C). Blood sugar is 200, taken 2 hours after breakfast. This is not satisfactory as regular insulin is quick acting and should have kept the postprandial blood at a lower level than 200.

Many more examples might be cited. A single blood sugar is always difficult to evaluate and repeated estimations must be made until the 24 hour pattern of that particular patient is established.

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THOMAS H. McGAVACK

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Dr. Waters has emphasized differences in behavior of simple sugars in blood. While much of what he has said falls far outside the daily routine of the clinician, many pertinent deductions of practical value can be drawn therefrom. For instance, the major portion of galactose must pass through the liver before it is made available in any quantity to function as blood sugar. This remains the fundamental fact of practical importance to the clinician in using some form of the galactose tolerance test for the diagnosis of disease. We prefer to use the intravenous test, which seems to give more reliable and more uniformly comparable results.

This galactose tolerance test proves of most value, as Dr. Waters has already emphasized, in diseases which may interfere with levels of blood glucose and liver function simultaneously. I refer especially to thyrotoxicosis and diabetes mellitus, particularly the form of diabetes mellitus that may be associated with a fatty liver or with diffuse pancreatic disease such as hemochromatosis. The intravenous administration of galactose in such instances and its subsequent periodic determination in the blood affords an accurate gauge of the glycogenic and possibly also of the glycogenolytic function of the liver.

In connection with Dr. Waters' comment on the behavior of the various simple sugars, one question seems apropos in conjunction with his remarks, and I quote: "While fructose will prevent the decrease in the blood glucose concentration of the liverless animal, there is no increase in the glucose concentration even when large doses of fructose are injected." Does Dr. Waters mean that under such circumstances the muscles and other tissues utilize fructose directly for energy and possible glycogen formation?

Concerning the methods for determining blood sugar, Dr. Waters has stressed those procedures which depend upon copper salts as the oxidizing agents. Does he by implication or otherwise feel that these are in general more accurate than those methods that depend upon the ferricyanide reaction? While we use the copper salt methods exclusively in our laboratories, there are certain modifications of the ferricyanide method such as the "timing method of Van Slyke and Hawkins" that are attractive because of the speed with which they can be performed.

I believe it is usually conceded that glutathione and ergothionine make up the greatest portion of the non-glucose reducing agents while creatinine, uric acid and some undetermined materials the remainder. Inasmuch as Benedict's copper sulfate reagent is probably not reduced by glutathione, this reagent should give rather more accurate results than other reagents. This I believe

is a fairly well settled matter but still one occasionally sees the statement questioned in the literature.

It is certainly important to touch upon the difference between the true blood sugar and all the reducing substances, especially in view of the fact that Mosenthal's extensive studies have demonstrated non-glucose reducing substances in quantities all the way from 1 mg. to 78 mg., with nearly 40 per cent of his subjects showing values above the ordinarily accepted maximum figure of 30 mg. per 100 cc. of blood. Inasmuch as the amount of non-glucose reducing substance may vary from time to time, even within a short space of time, we believe several specimens should be examined in doubtful cases, and the results of fermentation procedures compared with those in which copper salts are reduced. I hope that Dr. Mosenthal will amplify these points himself, so I shall not dwell further upon them.

It seems to us that in any discussion of methods for blood sugar, the problem actually begins, not in the laboratory, but at the bedside. The first point in this regard concerns a dictum rarely stressed, and, I must admit, often violated in our own work. It has been shown that the insertion of a needle into a vein may in a reasonable time, five minutes or so, produce a variation in the value for blood sugar. This affords plenty of time for withdrawing satisfactory specimens. However, usually a tourniquet is used. Within 15 to 30 seconds, this may cause fluctuations, commonly up, but sometimes down, in the value for blood sugar from 15 to 25 mg. per 100 cc. of blood. It seems an important rule, therefore, never to apply the tourniquet until completely in readiness. If venipuncture is unsuccessful at the end of a maximum of 15 seconds, it then should be removed and the procedure tried on the opposite arm.

The next point in obtaining the sample to be tested concerns the timing of our specimen. I shall refer particularly to the diabetic in whom frequent determinations may be desirable. Our aim is to obtain as much information as possible about the condition of the subject with least disturbance to him. Diagnostic punctures are probably best made in the post-absorptive state, fasting. Further punctures are not necessary in our opinion until the patient becomes aglycosuric. As soon as this occurs both fasting and two and one-half hour post-prandial determinations are desirable to determine both the resting state and the response to the ingestion of blood sugar precursors. Thereafter the post-prandial value gives us an idea of the renal threshold for glucose and serves as a guide to further therapy. As a general rule the older diabetic may show a glucose value at this time up to 250 mg., and sometimes much higher without a spill of sugar in the urine. Further more, the post-prandial figures become both more desirable and valuable, because they can be obtained without disturbing the patient's therapeutic regimen in any way. Perhaps it is trite and forthright insulting to emphasize this really obvious point among physiologists and clinicians, but it is amazing how slow we have been—and I am certainly among the guilty ones—to urge the adoption of this change in routine upon both clinics and hospitals. It may be equally out of place to emphasize the fact that comparative glucose tolerance tests have no part in the management of the known diabetic. However, all too frequently we see such tests ordered. Moreover, comparative tests are of little value unless the patient is on a diet of fixed proportions and amounts of carbohydrate, protein and fat.

Dr. Waters has referred to the effect of fructose absorption upon the concentration of glucose in the blood. In the type of experiment described, does fructose go directly to a levulose furane form or via glucose? I assume the former. Since insulin is necessary for this effect of intravenously administered fructose in lowering the glucose of the blood, a possible explanation may be in the behavior of the fructose and glycogen. The former via a furane levulose form may enter directly into the three carbon stage as described by Cori, Stettin and others, thus never becoming available as glucose. Simultaneously, glycogenolysis may be depressed thus eliminating another source of glucose.

Dr. Waters has touched upon the ability

of the kidney to form glucose, a feat which it seems to accomplish not only from carbohydrate intermediaries but also from amino acids. While, in relation to the total glucose of blood and tissue this function may be small, in some experiments kidney slices in serum have elaborated more glucose than simultaneously prepared and treated liver slices. In any event, the question of the role of the kidney in the regulation of blood sugar deserves consideration. For instance, is it possible that the kidney has played a role in making a portion of the ingested galactose available to the peripheral tissues in the hepatectomized animals mentioned by Dr. Waters? In regard to this effect upon glucose formation, is the kidney subject to internal regulation of an endocrine nature not unlike that of the adrenal cortex? Selye's experimental work may suggest this. He has shown that, at least histologically, the kidney can be transformed experimentally into a structure of wholly endocrine type resembling the adrenal in appearance and lacking all excretory function.

Finally, there is another point at which Dr. Waters' comments may touch upon the endocrine system. He has mentioned the hyperglycemic factor found even in highly purified preparations of insulin. Does he think this factor plays any part in the production of the clinical state of "insulin resistance"? In view of the short duration of its action, we lean somewhat away from such an hypothesis. However, as insulin formation may be a continuous process, it seems a difficult one to abandon entirely.

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Charles H. Best

Professor of Physiology and Director of the Banting and Best Department of Medical Research, University of Toronto

I have been greatly impressed this evening by the familiarity which our clinical friends have demonstrated in the fields of physiology and biochemistry.

I am afraid that Dr. McGavack has posed so many questions to Dr. Waters that another full lecture may be required to answer them.

The President of the American Diabetes Association has shown himself familiar with all the steps in the oxidation of sugar and the formation of glycogen. I suspect that he has learned these rather recently, and many of us are in the same situation.

In connection with Dr. Allen's remarks, I may say, as I have said before, that his book on diabetes was something of a Bible to us in the early days of insulin, and I pay tribute to him as a great clinician, as well as a very productive physiologist and biochemist. It is a great pleasure to see those healthy looking patients of his.

I was at a medical meeting in Toronto not long ago. A young lady, daughter of a close friend of mine, gave a paper. It so happened that some years ago I lent her father a hand in teaching her to ride and also attended many piano recitals at which she was one of the performers. When I was suddenly asked to discuss her paper at the scientific meeting, I confess I could barely remember what it was about as I was so relieved she had not fallen off or played a wrong note.

Of course, I had no apprehension about Dr. Waters, a senior member of my staff who has always handled himself well, but I do confess that I was somewhat relieved when he stopped telling Welsh jokes and really got down to the subject of the paper of the evening.

I have just returned from the Federation meetings in Atlantic City and I can tell you that I listened to all the papers in two sections. I was chairman of both these sections. I could not get out.

One of the communications was on blood sugar and the young lady who gave the paper was challenged by a member of the audience, who turned out to be Dr. Somogyi.

He asked her about the value which she got. Her reply was really quite adequate. She said, "Sir. I used your recent method and followed it most carefully." There was no further comment.

One of the papers was by Dr. Reinecke, who spoke on the formation of sugar by the kidney. When this process is studied in the intact or hepatectomized animal, there is a definite quantity of sugar formed by the kidney which is however small when compared to that produced by the liver. There seems to be no doubt about the fact that the kidney is capable of gluconeogenesis. The stage has not been reached yet when the influence of the various hormones on this action of the kidney can be studied with much hope of success.

Since the war many of us have been taking stock of the situation in the treatment of diabetes and there are some points on the credit side and some that are not so satisfactory. The mortality statistics have on the whole been better than one would have predicted. On the other hand, many cases in which complications have occurred have been recorded. We are all grateful that more accurate knowledge of the situation has been secured. We realize, however, that much better clinical methods of treatment can be more generally applied. The experimentalist has fallen short of his goal in that he has not made available the best form of insulin, i.e. one which will be liberated in response to need. It is a terribly perplexing situation when a clinician states that he sees just as many complications with the accurate control of blood sugar as he does when the blood sugar in this patient is not accurately regulated. I must say that in many cases the comparison is between poor treatment and moderately good treatment and that the real physiological control of blood sugar is extremely difficult and perhaps at present unattainable in some young children.

On the other hand, Dr. Allen and others tell us that it is possible in many, perhaps in all of their cases, to secure accurate control and we must watch these patients very carefully. They may lead the way to better treatment of diabetes. Those of us who have worked on the experimental aspects of diabetes feel a great continuing responsibility to attempt to put into the hands of the clinicians the very best tools which can be made available.

We did of course follow a few adult diabetic dogs through five or six years of their lives after insulin became available, and there were few or no complications. We have not as yet followed the puppies, in which the pancreas has been removed, throughout the whole span of their lives, and I hope that this will now be done in many laboratories. If it can be done successfully in puppies and in other species, one will feel fairly certain that similar results can be secured in human subjects. We will be greatly encouraged.

It is very refreshing that a number of groups of experimentalists are studying atherosclerosis. Those of us who are particularly interested in this subject are very pleased that workers can produce cholesterol atherosclerosis in other species than in the rabbit when the basal metabolism is lowered by thyroidectomy or thiouracil. It is extremely interesting that atherosclerosis occurs so frequently in certain species in which the blood fats are normally at a high level. I think that great progress will come in this sphere.

I may predict also that there will be better forms of insulin; that the diet for diabetics will be improved from the point of view of protection against atherosclerosis, and if research workers are permitted to carry on with their peace-time interests, that there will be a still more rapid increase in the rate at which new and useful knowledge in the whole field of diabetes will accumulate.

LIBRARY NOTES

Address of Acceptance and Appreciation of the Gift of The Edwin Smith Papyrus

George Baehr

President, The New York Academy of Medicine

The Edwin Smith Papyrus is priceless. To quote the great Egyptologist, James Henry Breasted, it is "the oldest nucleus of really scientific medical knowledge in the world." Although its age is more than 3500 years, it is actually a reprint or transcription of an original medical treatise which, from certain words and idioms, can be dated by Egyptologists as having been written a thousand or more years previously.

Almost at the very dawn of recorded Egyptian civilization, perhaps about the time of the Old Kingdom, 5000 years ago when the Pyramids were being erected, a great physician wrote the text of a medical treatise with attention to clinical observation, diagnosis, prognosis and treatment. The Papyrus which has come to us, deals entirely with surgery in a series of forty-eight illustrative cases and it obviously records only part of the original work of the master. His knowledge of anatomy was founded on human dissection and he already knew that blood flowed from the heart through the blood vessels to all parts of the body.

So great was the professional authority of that ancient physician and teacher, that his works continued to be a scientific guide for medical practice for several millenniums in that pre-Hellenic era, as did later the precepts of Galen for another thousand years in the beginning of the Christian era. As Breasted has speculated, he may well have been the original father of medicine, Imhotep, who was later defied by the ancient Greeks as Asclepios (Aescalapius) and from whose teachings Hippocrates derived much of his knowledge of the arts and philosophy of medicine.

The officers and fellows of The New York Academy of Medicine are overwhelmed by the generosity of our great sister institutions, The Brooklyn Museum and the New-York Historical Society. Words cannot express the full measure of our indebtedness. The Edwin Smith Papyrus will be the jewel of our great medical library and a continuing inspiration to scientists and scholars the world over.

In presenting this historic manuscript to The New York Academy of Medicine, I am sure the donors were moved by the fact that the Academy's library has grown during more than a hundred years to be one of the greatest depositories of medical and related scientific literature in existence. Like the famous library of Alexandria before its destruction by the barbarians, it serves today as the very fountainhead of scientific research and medical education. Its 275,000 bound volumes, innumerable pamphlets and more than 2000 current medical and related scientific journals con-

* Given December 2, 1948 at The New York Academy of Medicine.

tain the past and present medical literature of all the nations of the globe. These vast resources of knowledge and learning are freely available not only to all physicians and students but also to the general public as an integral part of the New York Public Library System. It is one of the Academy's essential contributions to the public welfare.

The Rare Book Room of our library, where the Edwin Smith Papyrus will have its permanent place of honor, contains a remarkable collection of old books and ancient hand-illuminated manuscripts through which the evolution of the medical sciences can be traced back to remote antiquity. But lest there be a misunderstanding, let me state that this rare book collection has not been assembled merely to serve as an antiquarian's paradise. It is in fact the cornerstone of medical science and medical culture, the source of knowledge from which modern medicine has been derived.

We must confess to a deep sense of satisfaction at the confidence in the Academy which you, Mr. Sidney Davidson and you, Dr. Fenwick Beekman, and the institutions which you head, have demonstrated by entrusting this historic document to our care. We accept it as a public trust with a profound appreciation of the enormous responsibility. Our gratitude shall not be expressed in utterances of the moment but by the earnest manner in which we shall forever treasure this precious possession and shall make it freely available to the students and scholars of this country and of the world.

RECENT ACCESSIONS TO THE LIBRARY

("Possession does not imply approval.")

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